8/17/05

3/3,K,AB/23
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 2689698 IFI Acc No: 9603417
Document Type: C
SYNTHETIC PEPTIDES DERIVED FROM VITRONECTIN AND PHARMACEUTICAL COMPOSITIONS COMPRISING THEM; MODULATE ACTIVITY OF **PLASMINOGEN ACTIVATOR** INHIBITOR-1
Inventors: Shaltiel Shmuel (IL)
Assignee: Yeda Research & Development Co Ltd IL Assignee Code: 93576
Patent (No,Date), Applic (No,Date)
US 5491129 19960213 US 9398005 19930729
Calculated Expiration: 20130729
Priority Applic(No,Date): IL 102688 19920730; IL 104296 19921231

Abstract:
The invention relates to synthetic peptides derived from the K348-A380 (8-40 of SEQ ID NO:1) sequence of the vitronectin molecule. The peptides modulate the biological activities of **plasminogen activator** inhibitor-1 (PAI-1) and are useful as active ingredients of pharmaceutical compositions for the treatment of disorders such as bleeding disorders, acute myocardial infarction, deep vein thrombosis, pulmonary embolism, disseminated intravascular **coagulation**, tumor cell invasion and metastasis, inflammation, liver diseases, bacterial blood infections, pregnancy toxicosis, and pathological conditions associated with the control of angiogenesis, or with nerve regeneration, or with excessive

4/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10085440 97407955

Characterization of the precursor of **prostate-specific antigen**. Activation by trypsin and by human glandular kallikrein.

Takayama TK; Fujikawa K; Davie EW
Department of Biochemistry, University of Washington, Box 357350, Seattle, WA 98195-7350, USA.

Journal of biological chemistry (UNITED STATES) Aug 22 **1997**, 272 (34) p21582-8, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: HL16919, HL, NHLBI; DK02447, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The precursor or zymogen form of **prostate-specific antigen** (pro-**PSA**) is composed of 244 amino acid residues including an amino-terminal propiece of 7 amino acids. Recombinant pro-**PSA** was expressed in Escherichia coli, isolated from inclusion bodies, refolded, and purified. The zymogen was readily activated by trypsin at a weight ratio of 50:1 to generate **PSA**, a serine protease that cleaves the chromogenic chymotrypsin substrate 3-carbomethoxypropionyl-L-arginyl-L-prolyl-L-**tyrosine** -p-nitroanili ne- HCl (S-2586). In this activation, the amino-terminal propiece Ala-Pro-Leu-Ile-Leu-Ser-Arg was released by cleavage at the Arg-Ile peptide bond. The recombinant pro-**PSA** was also activated by recombinant human glandular kallikrein, another prostate-specific serine protease, as well as by a partially purified protease(s) from seminal plasma. The recombinant **PSA** was inhibited by alpha1-antichymotrypsin, forming an equimolar complex with a molecular mass of approximately 100 kDa. The recombinant **PSA** failed to activate single chain urokinase-type plasminogen activator, in contrast to the recombinant hK2, which readily activated single chain urokinase-type plasminogen activator. These results indicate that pro-**PSA** is converted to an active serine protease by minor proteolysis analogous to the activation of many of the proteases present in blood, pancreas, and other tissues. Furthermore, **PSA** is probably generated by a cascade system involving a series of precursor proteins. These proteins may interact in a stepwise manner similar to the generation of plasmin during fibrinolysis or thrombin during blood coagulation.

Characterization of the precursor of **prostate-specific antigen**. Activation by trypsin and by human glandular kallikrein.

Aug 22 **1997**,

The precursor or zymogen form of **prostate-specific antigen** (pro-**PSA**) is composed of 244 amino acid residues including an amino-terminal propiece of 7 amino acids. Recombinant pro-**PSA** was expressed in Escherichia coli, isolated from inclusion bodies, refolded, and purified. The zymogen was readily activated by trypsin at a weight ratio of 50:1 to generate **PSA**, a serine protease that cleaves the chromogenic chymotrypsin substrate 3-carbomethoxypropionyl-L-arginyl-L-prolyl-L-**tyrosine** -p-nitroanili ne- HCl (S-2586). In this activation, the amino-terminal propiece Ala-Pro ...

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PSA was inhibited by alpha1-antichymotrypsin, forming an equimolar complex with a molecular mass of approximately 100 kDa. The recombinant **PSA** failed to activate single chain urokinase-type plasminogen activator, in contrast to the recombinant hK2, which readily activated single chain urokinase-type plasminogen activator. These results indicate that pro-**PSA** is converted to an active serine protease by minor proteolysis analogous to the activation of many of the proteases present in blood, pancreas, and other tissues. Furthermore, **PSA** is probably generated by a cascade system involving a series of precursor proteins. These proteins...

Descriptors: Kallikreins--Metabolism--ME; ***Prostate-Specific Antigen**--Metabolism--ME; *Trypsin--Metabolism--ME

...Enzyme No.: 3.4.21.- (Kallikreins); EC 3.4.21.4 (Trypsin); EC 3.4.21.77 (**Prostate-Specific Antigen**)

Chemical Name: Kallikreins; (Trypsin; (**Prostate-Specific Antigen**; (Enzyme Precursors; (Recombinant Proteins

4/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10081451 97331384

A simple method for the isolation and culture of epithelial and stromal cells from benign and neoplastic prostates.

Krill D; Shuman M; Thompson MT; Becich MJ; Strom SC

Department of Cellular and Molecular Pathology, University of Pittsburgh Medical School, Pennsylvania, USA.

Urology (UNITED STATES) Jun **1997**, 49 (6) p981-8, ISSN 0090-4295
Journal Code: WSY

Languages: ENGLISH

Document type: JOURNAL ARTICLE

OBJECTIVES: Current primary prostate cell culture techniques use an overnight digestion or extensive media preparation. In this report, we describe a method for the culture of benign and neoplastic cells from human prostatectomy specimens that is rapid and contains no undefined factors in the medium. METHODS: Characterization of the human cultured prostate cells was performed using immunohistochemical methods and monoclonal antibodies AE1/AE3 and cytokeratin 8, as well as monoclonal antibodies against **prostate-specific antigen** (**PSA**). Polymerase chain reaction was used to measure the exclusive epithelial and stromal cell products, c-met and hepatocyte growth factor (HGF), respectively. Electron microscopy was performed to assess the cell junctions and morphologic features of epithelial cells. Optimum cell growth in different media was tested using a cell replication assay. RESULTS: Microscopic evidence revealed that the cells demonstrate typical epithelial morphology, with polyhedral cells forming tight junctions in a continuous monolayer. Desmosomes were present in electron micrographs of epithelial cells. The cultured epithelial cells described in this report also demonstrate positive cytokeratin staining. The epithelial cells reacted positively with **PSA** antibody, indicating that the cells retain their secretory role in cell culture for a limited period. Epithelial cells expressed the HGF receptor, c-met; stromal cells secreted HGF. Insulin, transferrin, and selenium increased the growth of cells in the chemically defined media, compared with minimum essential media (MEM) and Ham's F12. CONCLUSIONS: In summary, essentially pure cultures of prostate stromal or epithelial cells have been established using simple isolation and culture methods. These cells will be useful for the investigation of related growth factors, such as insulin-like growth factor I and insulin-like growth factor II, and in understanding the basis for stromal-epithelial cell interactions.

Jun **1997**,

... methods and monoclonal antibodies AE1/AE3 and cytokeratin 8, as well as monoclonal antibodies against **prostate-specific antigen** (**PSA**). Polymerase chain reaction was used to measure

the exclusive epithelial and stromal cell products, c...

...described in this report also demonstrate positive cytokeratin staining. The epithelial cells reacted positively with **PSA** antibody, indicating that the cells retain their secretory role in cell culture for a limited...
...; Techniques; Epithelium--Metabolism--ME; Epithelium--Pathology--PA; Hepatocyte Growth Factor--Biosynthesis--BI; Immunohistochemistry; Receptor Protein-**Tyrosine** Kinases--Biosynthesis--BI; Stromal Cells--Metabolism --ME
Enzyme No.: EC 2.7.11.- (Proto-Oncogene Protein c-met); EC 2.7.11.- (Receptor Protein-**Tyrosine** Kinases)
Chemical Name: Proto-Oncogene Protein c-met; (Receptor Protein-**Tyrosine** Kinases; (Hepatocyte Growth Factor

4/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10075110 97205638
Tissue expression of neu differentiation factor/heregulin and its receptor complex in prostate cancer and its biologic effects on prostate cancer cells in vitro.
Lyne JC; Melhem MF; Finley GG; Wen D; Liu N; Deng DH; Salup R
Division of Urology, University of Pittsburgh, Pennsylvania, USA.
cancer journal from Scientific American (UNITED STATES) Jan-Feb **1997**, 3 (1) p21-30, ISSN 1081-4442 Journal Code: CR8
Languages: ENGLISH
Document type: JOURNAL ARTICLE
BACKGROUND: Prostate cancer is the most common cancer in American men and the second leading cause of cancer death. All clinical observations correlate poorly differentiated high-grade prostate cancer with disease-specific mortality. The HER2 cell membrane **tyrosine** kinase, a member of the epidermal growth factor receptor family that is the transcription product of the erbB2neu oncogene, and HER3, a receptor protein of the same family, are overexpressed in prostate cancer and prostatic intraepithelial neoplasia. The ligand for these receptors and another related family member, HER4, has recently been identified by independent investigator groups and called neu differentiation factor (NDF) or heregulin. In vitro treatment of HER2- and HER3- or HER2- and HER4-expressing breast cancer cells stimulates **tyrosine** phosphorylation of HER2 and produces changes in the rate of proliferation, degree of cellular differentiation, and synthesis of physiologic secretion products. There are no published reports on the expression of NDF and HER4 in prostate cancer or the in vitro effects of NDF in prostate cancer cells. METHODS: Expression of NDF, HER2, HER3, and HER4 was studied in 24 frozen prostatectomy specimens by immunohistochemistry. The biologic effect of human recombinant NDF was studied in vitro, using the LNCaP, PC3, and DU145 human prostate cancer cell lines. HER and NDF protein expression was studied by immunohistochemistry. NDF mRNA was analyzed using reverse transcriptase polymerase chain reaction from whole RNA. The biologic effects of NDF on prostate cancer cells in vitro included cell proliferation, thymidine synthesis, induction of **prostate-specific antigen** mRNA, anchorage-dependent and anchorage-independent cell growth, and ploidy analysis. Data analysis was performed using Student's t test. RESULTS: Observations in clinical prostatectomy specimens: Immunohistochemistry studies in clinical prostatectomy specimens demonstrate absence of significant NDF expression in prostate cancer, whereas it is expressed in 100% of the stroma, 100% of basal epithelial cells, and 58% of luminal cells in normal and benign hyperplastic prostatic tissue. The HER4 receptor protein is strongly expressed by normal prostate luminal cells, but not prostate cancer. Benign prostate tissue exhibits strong expression of HER2, HER3, and HER4 by basal cells, but only luminal cells significantly express HER4. Only 23% of prostate cancer specimens express HER4, while 95% express HER3 and 82%

HER2. Prostatic intraepithelial neoplasia stained similarly to cancer for all proteins studied. Observations in prostate cancer cell lines: In vitro treatment with NDF significantly reduces aneuploidy and proliferation and growth of androgen-sensitive prostate cancer cells. Incubation with NDF also induces **prostate-specific antigen** mRNA in prostate cancer cells. In spite of displaying NDF mRNA, prostate cancer cells do not produce detectable NDF protein, but express HER2 and HER3 proteins. DISCUSSION: These data suggest that NDF may be a paracrine differentiation factor involved in normal adult prostate physiology and that functional loss of the NDF/HER ligand/ receptor loop may be an early event associated with prostate tumorigenesis.

Jan-Feb **1997**,

... correlate poorly differentiated high-grade prostate cancer with disease-specific mortality. The HER2 cell membrane **tyrosine** kinase, a member of the epidermal growth factor receptor family that is the transcription product...

... vitro treatment of HER2- and HER3- or HER2- and HER4-expressing breast cancer cells stimulates **tyrosine** phosphorylation of HER2 and produces changes in the rate of proliferation, degree of cellular differentiation...

... of NDF on prostate cancer cells in vitro included cell proliferation, thymidine synthesis, induction of **prostate-specific antigen** mRNA, anchorage-dependent and anchorage-independent cell growth, and ploidy analysis. Data analysis was performed...

... and proliferation and growth of androgen-sensitive prostate cancer cells. Incubation with NDF also induces **prostate-specific antigen** mRNA in prostate cancer cells. In spite of displaying NDF mRNA, prostate cancer cells do...

4/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09280517 97358621

PSA -NCAM and B-50/GAP-43 are coexpressed by specific neuronal systems of the adult rat mediobasal hypothalamus that exhibit remarkable capacities for morphological plasticity.

Alonso G; Prieto M; Legrand A; Chauvet N
INSERM U 336, University of Montpellier II, France.

Journal of comparative neurology (UNITED STATES) Jul 28 **1997**, 384 (2) p181-99, ISSN 0021-9967 Journal Code: HUV
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The present study was designed to determine whether the mediobasal hypothalamus of adult rats contains neurons that continue to coexpress the highly polysialylated neural cell adhesion molecule (**PSA**-NCAM) and B-50/GAP-43, two proteins coexpressed by virtually all of the neurons of the fetal and neonatal rat central nervous system. Confocal laser scanning microscopy combined with double- or triple-fluorescence immunostaining was used to identify the hypothalamic neurons that express high levels of both **PSA** -NCAM and B-50/GAP-43 and to study the possible modifications of their morphological organization following a surgical lesion through the mediobasal hypothalamus. In intact animals, **PSA**-NCAM and B-50/GAP-43 were found to be colocalized within numerous fibers projecting throughout the external layer of the median eminence that were immunoreactive for either gamma-aminobutyric acid (GABA) or **tyrosine** hydroxylase (TH). Three to 30 days after a lesion through this region, numerous regenerating axonal sprouts, triple-immunostained for **PSA**-NCAM, B-50/GAP-43, and either GABA or TH, were detected along the ventricular surface of, and throughout the perivascular layer of, the median eminence. Surprisingly, high levels of **PSA** -NCAM and B-50/GAP-43 were also associated with

numerous supraependymal neurons that exhibited long ramified processes and were immunoreactive for GABA but TH-negative. The use of the proliferation marker, 3H-thymidine, further indicated that the emergence of such supraependymal neurons after median eminence lesion was not related to the proliferation of preexisting quiescent cells. These data indicate that the mediobasal hypothalamus of the adult rat contains two neuronal systems, in which the continued coexpression of **PSA** -NCAM and B-50/GAP-43 is related to remarkable capacities for postlesional, morphological plasticity.

PSA -NCAM and B-50/GAP-43 are coexpressed by specific neuronal systems of the adult...
Jul 28 **1997**,
... adult rats contains neurons that continue to coexpress the highly polysialylated neural cell adhesion molecule (**PSA** -NCAM) and B-50/GAP-43, two proteins coexpressed by virtually all of the neurons...

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... hypothalamus of the adult rat contains two neuronal systems, in which the continued coexpression of **PSA**-NCAM and B-50/GAP-43 is related to remarkable capacities for postlesional, morphological plasticity.
...; Regeneration; Neurofilament Proteins--Biosynthesis--BI; Neurons --Ultrastructure--UL; Rats; Rats, Sprague-Dawley; Thymidine--Metabolism--ME ; **Tyrosine** 3-Monooxygenase--Metabolism--ME
Enzyme No.: EC 1.14.16.2 (**Tyrosine** 3-Monooxygenase)
Chemical Name: **Tyrosine** 3-Monooxygenase; (polysialyl neural cell adhesion molecule; (GAP-43 Protein; (Membrane Glycoproteins; (Nerve Tissue Proteins...

4/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09099053 97246572
Characterization of **prostate-specific antigen** proteolytic activity on its major physiological substrate, the sperm motility inhibitor precursor/semenogelin I.
Robert M; Gibbs BF; Jacobson E; Gagnon C
Urology Research Laboratory, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.
Biochemistry (UNITED STATES) Apr 1 **1997**, 36 (13) p3811-9, ISSN 0006-2960 Journal Code: A0G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The protease **prostate-specific antigen** (**PSA**) is a marker widely used clinically for monitoring prostatic malignancies. Under normal conditions, this enzyme is mainly involved in the post ejaculation degradation of the major human seminal protein, the seminal plasma motility inhibitor precursor/semenogelin I (SPMIP/SgI), which is the predominant

protein component of human semen coagulum. **PSA** primary structure and activity on synthetic substrates predict a chymotrypsin-like activity whose specificity remains to be established. The present study was aimed at characterizing the proteolytic processing of the SPMIP/SgI by **PSA**. Purified SPMIP/SgI was incubated with **PSA** in the presence or absence of protease inhibitors. General serine protease inhibitors, heavy metal cations (Zn2+ and Hg2+), and the heavy metal chelator 1,10-phenanthroline partially or totally inhibited the proteolytic activity of **PSA** toward SPMIP/SgI. Under identical conditions, other proteins, such as bovine serum albumin, ovalbumin, and casein, were very poor substrates for **PSA**. Hydrolysis products were separated by reverse-phase high-performance liquid chromatography, assayed for sperm motility inhibitory activity, and analyzed by immunoblotting and mass spectrometry. The region responsible for the sperm motility inhibitory activity and containing an SPMI antiserum epitope was localized to the N-terminal portion of the molecule between residues 85 and 136. On the other hand, a monoclonal antibody against a seminal vesicle-specific antigen (MHS-5) recognized fragments derived from the central part of the SPMIP/SgI (residues 198-223). **PSA** hydrolysis occurred almost exclusively at either leucine or **tyrosine** residues, demonstrating directly for the first time a restricted chymotrypsin-like activity on a physiological substrate. The results suggest that **PSA** is the main enzyme responsible for the processing of SPMIP/SgI in human semen and that this protease manifests unusual specificity with respect to hydrolyzable substrates and sites of hydrolysis.

Characterization of **prostate-specific antigen** proteolytic activity on its major physiological substrate, the sperm motility inhibitor precursor/semenogelin I.
Apr 1 **1997**,
The protease **prostate-specific antigen** (**PSA**) is a marker widely used clinically for monitoring prostatic malignancies. Under normal conditions, this enzyme...

... precursor/semenogelin I (SPMIP/SgI), which is the predominant protein component of human semen coagulum. **PSA** primary structure and activity on synthetic substrates predict a chymotrypsin-like activity whose specificity remains...

...The present study was aimed at characterizing the proteolytic processing of the SPMIP/SgI by **PSA**. Purified SPMIP/SgI was incubated with **PSA** in the presence or absence of protease inhibitors. General serine protease inhibitors, heavy metal cations...

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... 5) recognized fragments derived from the central part of the SPMIP/SgI (residues 198-223). **PSA** hydrolysis occurred almost exclusively at either leucine or **tyrosine** residues, demonstrating directly for the first time a restricted chymotrypsin-like activity on a physiological substrate. The results suggest that **PSA** is the main enzyme responsible for the processing of SPMIP/SgI in human semen and...
Descriptors: Prostate--Enzymology--EN; ***Prostate-Specific Antigen**--Metabolism--ME; *Protein Precursors--Metabolism--ME; *Proteins--Metabolism--ME; *Sex Hormones--Metabolism--ME
Enzyme No.: EC 3.4.21.1 (Chymotrypsin); EC 3.4.21.77 (**Prostate-Specific Antigen**)
Chemical Name: Chymotrypsin; (**Prostate-Specific Antigen** ; (semenogelin; (seminal plasma motility inhibitor; (Antibodies, Monoclonal ; (Peptide Fragments; (Phenanthrolines; (Protease Inhibitors; (Protein Precursors; (Proteins...

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? ds

Set     Items   Description
S1      24600   PROSTATE(W)SPECIFIC(W)ANTIGEN OR PSA
S2     217395   TYROSINE
S3        117   S1 AND S2
S4         84   S3 AND PY<1998
S5      10215   PRODRUG
? s factor(w)x

         1449851  FACTOR
         1443908  X
      S6    7660  FACTOR(W)X
? s s5 and s6

           10215  S5
            7660  S6
      S7       1  S5 AND S6
? t s7/3,k,ab/1


 7/3,K,AB/1     (Item 1 from file: 34)
DIALOG(R)File  34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

00560929   Genuine Article#: EC362   Number of References: 38
Title: PROTEOLYTIC ACTIVATION OF HUMAN FACTOR-IX AND FACTOR-X
    BY RECOMBINANT HUMAN FACTOR-VIIA - EFFECTS OF CALCIUM, PHOSPHOLIPIDS,
    AND TISSUE FACTOR
Author(s): KOMIYAMA Y; PEDERSEN AH; KISIEL W
Corporate Source: UNIV NEW MEXICO,SCH MED,DEPT PATHOL,BLOOD SYST RES FDN
    LAB/ALBUQUERQUE//NM/87131; UNIV NEW MEXICO,SCH MED,DEPT PATHOL,BLOOD
    SYST RES FDN LAB/ALBUQUERQUE//NM/87131; NOVO IND AS,NOVO RES
    INST/DK-2800 BAGSVAERD//DENMARK/
Journal: BIOCHEMISTRY, 1990, V29, N40, P9418-9425
Language: ENGLISH   Document Type: ARTICLE

Title: PROTEOLYTIC ACTIVATION OF HUMAN FACTOR-IX AND FACTOR-X
    BY RECOMBINANT HUMAN FACTOR-VIIA - EFFECTS OF CALCIUM, PHOSPHOLIPIDS,
    AND TISSUE FACTOR
Research Fronts: 88-0643 001   (LIPOPHILIC MITOMYCIN-C PRODRUG
    -BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)
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12/3,K,AB/3 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05900205 Genuine Article#: XF329 Number of References: 53
Title: Activation of blood coagulation **factor X** by **arginine**-specific cysteine proteinases (gingipain-Rs) from Porphyromonas gingivalis
Author(s): Imamura T; Potempa J; Tanase S; Travis J (REPRINT)
Corporate Source: UNIV GEORGIA,DEPT BIOCHEM/ATHENS//GA/30602 (REPRINT); UNIV GEORGIA,DEPT BIOCHEM/ATHENS//GA/30602; KUMAMOTO UNIV,GRAD SCH MED SCI, DEPT NEUROSCI & IMMUNOL, DIV MOL PATHOL/KUMAMOTO 860//JAPAN/; JAGIELLONIAN UNIV,INST MOL BIOL, DEPT IMMUNOL & MICROBIOL/PL-31120 KRAKOW//POLAND/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N25 (JUN 20), P 16062-16067
ISSN: 0021-9258 Publication date: 19970620
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: The effect of two **arginine**-specific cysteine proteinases (gingipain Rs) from Porphyromonas gingivalis, a causative bacterium of adult periodontitis, on human blood coagulation was investigated, Activated partial thromboplastin time and prothrombin time were shortened by these proteinases, with a 95-kDa gingipain R containing adhesin domains being B-fold more efficient in comparison to a 50-kDa gingipain R containing the catalytic domain alone, The 50-kDa enzyme reduced each coagulation time in several plasmas deficient in various coagulation factors, while it was ineffective in **factor X** -deficient plasma unless reconstituted with this protein. Each proteinase activated **factor X** in a dose- and time-dependent manner, with Michaelis constants (K-m) being found to be lower than the normal plasma **factor X** concentration, strongly suggesting that **factor X** activation by gingipain Rs, especially the 95-kDa form which is strongly activated by phospholipids, could occur in plasma. This is the first report of **factor X** activation by bacterial proteinases and indicates that the gingipain Rs could be responsible for the production of thrombin and, indirectly, with the generation of prostaglandins, interleukin-1, etc., which have been found to be associated with the development of periodontitis induced by P. gingivalis infections. Furthermore, the data support the hypothesis that induction of blood coagulation by bacterial proteinases may be a causative agent in the pathogenesis of disseminated intravascular coagulation in sepsis.

Title: Activation of blood coagulation **factor X** by **arginine**-specific cysteine proteinases (gingipain-Rs) from

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? ds

Set     Items   Description
S1      15549   PRODRUG??
S2       7660   FACTOR(W)X
S3         34   ARG?(5N)194
S4          0   S1 AND S3
S5         11   ARGININE(5N)194
S6          5   RD (unique items)
S7          0   S1 AND S6
? s arginine

     S8  132349  ARGININE
? s s2 and s8

           7660  S2
         132349  S8
     S9     196  S2 AND S8
? s tyrosine

    S10  217395  TYROSINE
? s s9 and s10

            196  S9
         217395  S10
    S11       7  S9 AND S10
? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
    S12       6  RD (unique items)
? t s12/3,k,ab/1-6


 12/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

08879549   97094811
  Factor VII central. A novel mutation in the catalytic domain that reduces
tissue factor binding, impairs activation by factor Xa, and abolishes
amidolytic and coagulant activity.
  Bharadwaj D; Iino M; Kontoyianni M; Smith KJ; Foster DC; Kisiel W
  Department of Pathology, University of New Mexico School of Medicine,
Albuquerque, New Mexico 87131, USA.
  Journal of biological chemistry (UNITED STATES)  Nov 29 1996,  271 (48)
p30685-91,  ISSN 0021-9258  Journal Code: HIV
  Contract/Grant No.: HL35246, HL, NHLBI
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  Factor VII is a vitamin K-dependent zymogen of a serine protease that
participates in the initial phase of blood coagulation. A factor VII
molecular variant (factor VII Central) was identified in a 24-year-old male
with severe factor VII deficiency and whose plasma factor VII antigen was
38% of normal, but expressed <1% factor VII procoagulant activity. DNA
sequence analysis of the patient's factor VII gene revealed a thymidine to
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cytidine transition at nucleotide 10907 in exon VIII that results in a novel amino acid substitution of Phe328 to Ser. The patient was homozygous for this mutation, whereas each parent of the patient was heterozygous for this mutation. To investigate the molecular properties of this variant, a recombinant F328S factor VII mutant was prepared and analyzed in relation to wild-type factor VII. F328S factor VII exhibited <1% factor VII procoagulant activity and a 2-fold decreased affinity for tissue factor and failed to activate **factor X** or IX in the presence of tissue factor following activation by factor Xa. In addition, F328S factor VIIa exhibited no detectable amidolytic activity in the presence of tissue factor. The rate of F328S factor VII activation by factor Xa was markedly decreased relative to the rate of wild-type factor VII activation as revealed by densitometry scanning of SDS gels. Temporal analysis of this reaction by SDS-polyacrylamide gel electrophoresis also revealed the formation of two novel F328S factor VII degradation products (40 and 9 kDa) resulting from factor Xa proteolysis of the Arg315-Lys316 peptide bond in intact F328S factor VII. Computer modeling and molecular dynamics simulations of the serine protease domain of factor VIIa suggested that the inability of F328S factor VIIa to cleave substrates may result from the apparent formation of a hydrogen bond between Tyr377 and Asp338, a residue at the bottom of the substrate-binding pocket important for the interaction of substrate **arginine** side chains with the enzyme. These findings suggest that Phe328, which is conserved in prothrombin, factor IX, **factor X**, factor VII, and trypsin, is important for factor VIIa catalysis.

... procoagulant activity and a 2-fold decreased affinity for tissue factor and failed to activate **factor X** or IX in the presence of

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 ds

Set     Items   Description
S1      15549   PRODRUG??
S2       7660   FACTOR(W)X
S3         34   ARG?(5N)194
S4          0   S1 AND S3
S5         11   ARGININE(5N)194
S6          5   RD (unique items)
S7          0   S1 AND S6
S8     132349   ARGININE
S9        196   S2 AND S8
S10    217395   TYROSINE
S11         7   S9 AND S10
S12         6   RD (unique items)
? s psa or prostate(w)specific(w)antigen

          16728  PSA
         103411  PROSTATE
        1645050  SPECIFIC
         614986  ANTIGEN
          18966  PROSTATE(W)SPECIFIC(W)ANTIGEN
     S13  24600  PSA OR PROSTATE(W)SPECIFIC(W)ANTIGEN
? s s2 and s13

           7660  S2
          24600  S13
     S14      4  S2 AND S13
? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S15      4  RD (unique items)
? t s15/3,k,ab/1-4


 15/3,K,AB/1     (Item 1 from file: 55)
DIALOG(R)File  55:Biosis Previews(R)
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12283799   BIOSIS NO.: 200000037301
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? ds

Set      Items   Description
S1        7660   FACTOR(W)X
S2      517277   MUTAT?
S3         491   S1 AND S2
S4      632588   ARG? OR TYR?
S5         160   S3 AND S4
S6         125   S5 AND PY<1998
S7      197407   CLEAV?
S8          54   S6 AND S7
S9      921467   ENZYME
S10         15   S8 AND S9
S11         10   RD (unique items)
? psa or prostate(w)specific(w)antigen

Ref   Items  Index-term
E1        1  SA NODE RHYTHMS
E2        1  SA NODE SINGLE CELLS
E3        0 *SA OR PROSTATE(W)SPECIFIC(W)ANTIGEN
E4        1  SA PREPOLYMER, CPP PREPOLYMER AND 20-80/CPP-SA
E5        1  SA PRIMARY AFFERENT
E6       14  SA PROTEIN
E7        1  SA SALICYLIC ACID
E8        1  SA SITE
E9        1  SA SODIUM-AZIDE
E10       1  SA STAPHYLOCOCCUS-AUREUS (MICROCOCCACEAE)
E11       1  SA UNIT-TYPE
E12       1  SA U1000 SPECTROMETER

          Enter P or PAGE for more
? s psa or prostate(w)specific(w)antigen

         16728  PSA
        103411  PROSTATE
       1645050  SPECIFIC
        614986  ANTIGEN
         18966  PROSTATE(W)SPECIFIC(W)ANTIGEN
    S12  24600  PSA OR PROSTATE(W)SPECIFIC(W)ANTIGEN
? s  s12 and s9

         24600  S12
        921467  S9
    S13   1028  S12 AND S9
? s s13 and s7

          1028  S13
        197407  S7
    S14     79  S13 AND S7
? s site??

    S15 1336861  SITE??
? s s14 and s15

            79  S14
       1336861  S15
    S16     27  S14 AND S15
```

? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
S17 18 RD (unique items)
? t s17/3,k,ab/1-18

17/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10130436 99247617
Kininogenase activity of prostate-derived human glandular kallikrein (hK2) purified from seminal fluid.
Charlesworth MC; Young CY; Miller VM; Tindall DJ
Department of Urology, Mayo Foundation, Rochester, Minnesota 55905, USA.
Journal of andrology (UNITED STATES) Mar-Apr 1999, 20 (2) p220-9,
ISSN 0196-3635 Journal Code: HB4
Contract/Grant No.: CA-70892, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Prostate-specific human glandular kallikrein (hK2) is an active **enzyme** in human seminal fluid. It is one of three serine proteases in the human kallikrein gene family, which includes hK1 (tissue kallikrein) and hK3 (**prostate-specific antigen** [**PSA**]). In order to examine kininogenase activity (i.e., production of kinin by these enzymes), we tested for bradykinin and/or Lys-bradykinin release upon incubation of hK2 and for other kallikreins with high-molecular weight kininogen (HMWK), which contains the nonapeptide bradykinin. Kinins are important regulatory peptides (especially for vascular permeability), and they may have a role in enhancing sperm motility. High-molecular weight kininogen is the substrate for plasma kallikrein (PKa potent kinin-generating **enzyme** circulating in blood, not of the same gene family) and for hK1. Glandular kallikrein and protein-C inhibitor (PCI)-hK2 complex, a serpin protease inhibitor that binds hK2, were purified to homogeneity by affinity and size-exclusion chromatography. About one-half of the hK2 is found in complex with PCI. The kallikrein enzymes were incubated with HMWK, and the resulting **cleavage** products were analyzed for kinin activity using **enzyme** immunoassay, high-performance liquid chromatography and mass spectrometry, and in vitro bioassay. Our results show that hK2 **cleaves** HMWK to produce bradykinin, not Lys-bradykinin (like hK1), and the resultant heavy (56-kDa) and light (42-kDa) chains of HMWK show similar electrophoretic mobility to those **cleaved** by PK. **Prostate-specific antigen** (hK3) had no kinin-generating activity. We also identified three other internal **cleavage sites** for hK2 in HMWK (Arg427, Arg437, and Arg457) that yielded two peptides, one of which is identical to a PK-**cleaved** peptide. Glandular kallikrein is about 500-fold less active than is PK or tissue kallikrein, but it may play a physiologically important role in bradykinin release in seminal fluid.

Prostate-specific human glandular kallikrein (hK2) is an active **enzyme** in human seminal fluid. It is one of three serine proteases in the human kallikrein gene family, which includes hK1 (tissue kallikrein) and hK3 (**prostate-specific antigen** [**PSA**]). In order to examine kininogenase activity (i.e., production of kinin by these enzymes), we...

... motility. High-molecular weight kininogen is the substrate for plasma kallikrein (PKa potent kinin-generating **enzyme** circulating in blood, not of the same gene family) and for hK1. Glandular kallikrein and...

... found in complex with PCI. The kallikrein enzymes were incubated with HMWK, and the resulting **cleavage** products were analyzed for kinin activity using **enzyme** immunoassay, high-performance liquid chromatography and mass spectrometry, and in vitro bioassay. Our results show that hK2 **cleaves** HMWK to produce bradykinin, not Lys-bradykinin (like hK1), and the resultant heavy (56-kDa) and light (42-kDa) chains of HMWK show similar electrophoretic mobility to those **cleaved** by PK. **Prostate-specific antigen** (hK3) had no kinin-generating activity. We also identified three other internal **cleavage sites** for hK2 in HMWK (Arg427, Arg437, and Arg457) that yielded two peptides, one of which is identical to a PK-**cleaved** peptide. Glandular kallikrein is about 500-fold less active than is PK or tissue kallikrein...

17/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10124879 99155098
cDNA cloning and molecular characterization of human brain metalloprotease MP100: a beta-secretase candidate?
Huber G; Thompson A; Gruninger F; Mechler H; Hochstrasser R; Hauri HP; Malherbe P
Pharma Division, Preclinical CNS Research, F. Hoffmann-La Roche Ltd., Basel, Switzerland.
Journal of neurochemistry (UNITED STATES) Mar 1999, 72 (3) p1215-23, ISSN 0022-3042 Journal Code: JAV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Metalloprotease MP100 was originally isolated as a beta-secretase candidate from human brain using a beta-amyloid precursor protein (beta-APP)-derived p-nitroanilide (pNA) peptide substrate. Peptide sequences from purified MP100 were now found to resemble sequences reported for a puromycin-sensitive aminopeptidase (**PSA**) highly enriched in brain, and cDNA cloning revealed nearly complete homology of MP100 to **PSA** , with only a single bp difference resulting in an amino acid change at position 184. Another MP100 cDNA encoded a protein with a 36-amino acid deletion (positions 180-217) and a two-amino acid insertion after Val533. Purified recombinant human MP100 **cleaved** the original pNA substrate as well as a free beta-**site**-spanning amyloid beta (A beta) peptide (A beta(-10/+10)), generating A beta(1-10). The latter substrate, however, remained uncleaved, if N- and C-terminally blocked, and also purified beta-APP was not **cleaved**. Double immunoimaging revealed partial, patchy, colocalization of beta-APP and MP100 in doubly transfected human embryonic kidney cells (HEK cells) and in normal neuroblastoma cells, and both proteins could be coimmunoprecipitated from rat brain extracts, suggesting their close vicinity in vivo. Coexpression of MP100 and beta-APP695, however, did not boost A beta levels in HEK cells, although active **enzyme** was produced. Thus, MP100 does not exert true beta-secretase-like function in cells, although it may well act as a secondary exoprotease in a complex beta-APP/A beta metabolism.

... from purified MP100 were now found to resemble sequences reported for a puromycin-sensitive aminopeptidase (**PSA**) highly enriched in brain, and cDNA cloning revealed nearly complete homology of MP100 to **PSA**, with only a single bp difference resulting in an amino acid change at position 184...

...positions 180-217) and a two-amino acid insertion after Val533. Purified recombinant human MP100 **cleaved** the original pNA substrate as well as a free beta-**site** -spanning amyloid beta (A beta) peptide (A beta(-10/+10)), generating A beta(1-10...

... remained uncleaved, if N- and C-terminally blocked, and also purified

beta-APP was not **cleaved** . Double immunoimaging revealed partial, patchy, colocalization of beta-APP and MP100 in doubly transfected human...

... and beta-APP695, however, did not boost A beta levels in HEK cells, although active **enzyme** was produced. Thus, MP100 does not exert true beta-secretase-like function in cells, although...
...; beta-Protein Precursor--Metabolism--ME; Base Sequence; Cell Line; Cloning, Molecular; DNA, Complementary--Genetics--GE; **Enzyme**-Linked Immunosorbent Assay; Fluorescent Antibody Technique, Indirect; Kidney --Metabolism--ME; Molecular Sequence Data; Precipitin Tests...

17/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10082592 97352541
Ala217 is important for the catalytic function and autoactivation of prostate-specific human kallikrein 2.
Mikolajczyk SD; Millar LS; Marker KM; Grauer LS; Goel A; Cass MM; Kumar A ; Saedi MS
Hybritech Incorporated, San Diego, California 92196-9006, USA. sdmikolajczyk@beckman.com
European journal of biochemistry (GERMANY) Jun 1 1997, 246 (2) p440-6, ISSN 0014-2956 Journal Code: EMZ
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Prostate-specific human kallikrein, hK2, is a serine protease found in prostate tissues that has 78% amino acid sequence identity with **prostate-specific antigen** (**PSA**). We have previously reported the affinity purification of hK2 heterologously expressed in a hamster cell line and demonstrated an arginine-restricted substrate specificity. Here, we describe the cloning, expression, purification, and enzymatic activity of a mutant form of hK2 containing an alanine to valine substitution at residue 217 ([Val217]hK2). This mutant form was secreted into the serum-free spent media of recombinant cells as the stable proenzyme form ([Val217]phK2). Mild trypsin treatment was used to convert [Val217]phK2 to the active form, which had reduced catalytic function compared to the wild-type hK2. Kinetic studies using the chromogenic substrate D-H-Pro-Phe-Arg-4-nitroanilide showed that [Val217]hK2 has significantly decreased substrate binding, with a K(m) of 4200 microM compared to 11 microM for wild-type hK2. The k(cat) for [Val217]hK2 was more than 100-fold lower than for hK2. hK2, but not [Val217]hK2, was able to activate [Val217]phK2. [Val217]hK2 also showed altered specificity on a synthetic peptide substrate compared to wild-type hK2, which exhibited partial hydrolysis at a **PSA** chymotrypsin-like **cleavage site** as well as the trypsin-like **site cleaved** by hK2. These results indicate that Ala217 is a key residue affecting the catalytic properties of hK2.

... a serine protease found in prostate tissues that has 78% amino acid sequence identity with **prostate-specific antigen** (**PSA**). We have previously reported the affinity purification of hK2 heterologously expressed in a hamster cell...

...a synthetic peptide substrate compared to wild-type hK2, which exhibited partial hydrolysis at a **PSA** chymotrypsin-like **cleavage site** as well as the trypsin-like **site cleaved** by hK2.
These results indicate that Ala217 is a key residue affecting the catalytic properties...
; Amino Acid Sequence; Base Sequence; Catalysis; Cell Line; Cloning, Molecular; DNA, Complementary; **Enzyme** Activation; Hamsters; Kallikreins--Genetics--GE; Kallikreins--Isolation and Purification--IP; Kinetics; Molecular Sequence Data; Mutagenesis

17/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09342499 98063145

Prostate-specific antigen forms complexes with human alpha 2-macroglobulin and binds to the alpha 2-macroglobulin receptor/LDL receptor-related protein.

Otto A; Bar J; Birkenmeier G
Institute of Biochemistry, University of Leipzig, Germany.

Journal of urology (UNITED STATES) Jan 1998, 159 (1) p297-303, ISSN 0022-5347 Journal Code: KC7
Languages: ENGLISH
Document type: JOURNAL ARTICLE

PURPOSE: To investigate the binding of the **prostate-specific antigen** (**PSA**) to human alpha 2-macroglobulin (alpha 2-M) and to alpha 1-antichymotrypsin (ACT). MATERIALS AND METHODS: Binding analysis was evaluated by electrophoresis, Western-blotting, **enzyme** -linked immunosorption assay (ELISA) and size exclusion chromatography. Quantification of **PSA** and of different forms of alpha 2-M was performed using commercial test kits. The **cleavage site** of **PSA** in alpha 2-M was analyzed by SDS-PAGE and microsequencing. RESULTS: Binding of **PSA** to alpha 2-M is initiated by the **cleavage** of the peptide bond between amino acids Tyr 686 and Glu 687 of the bait region indicating a chymotrypsin-like activity of the **PSA** . The **PSA**'s proteolytic **cleavage** triggers the transformation of alpha 2-M as detected by conformation-specific monoclonal antibodies. Kinetic analysis revealed faster binding of **PSA** to alpha 2-M than to ACT. The **PSA** bound to alpha 2-M is caged by the inhibitor and thus escapes detection by antibodies. This results in an incorrect calculation of the level of **PSA** when released from prostate into the blood. Complexes of **PSA**-alpha 2-M and **PSA**-ACT were found to bind to the alpha 2-macroglobulin receptor/LDL receptor-related protein (alpha 2-M-R/LRP) which may be the clearance receptor for **PSA**. CONCLUSIONS: Quantifying free **PSA** and **PSA**-ACT complexes, as routinely done in managing prostate-associated diseases, does not represent the total secretion capacity of the prostate. The proteinase inhibitor alpha 2-M has to be considered as a main contributor to **PSA** complex formation in the blood.

Prostate-specific antigen forms complexes with human alpha 2-macroglobulin and binds to the alpha 2-macroglobulin receptor...

PURPOSE: To investigate the binding of the **prostate-specific antigen** (**PSA**) to human alpha 2-macroglobulin (alpha 2-M) and to alpha 1-antichymotrypsin (ACT). MATERIALS AND METHODS: Binding analysis was evaluated by electrophoresis, Western-blotting, **enzyme** -linked immunosorption assay (ELISA) and size exclusion chromatography. Quantification of **PSA** and of different forms of alpha 2-M was performed using commercial test kits. The **cleavage site** of **PSA** in alpha 2-M was analyzed by SDS-PAGE and microsequencing. RESULTS: Binding of **PSA** to alpha 2-M is initiated by the **cleavage** of the peptide bond between amino acids Tyr 686 and Glu 687 of the bait region indicating a chymotrypsin-like activity of the **PSA** . The **PSA**'s proteolytic **cleavage** triggers the transformation of alpha 2-M as detected by conformation-specific monoclonal antibodies. Kinetic analysis revealed faster binding of **PSA** to alpha 2-M than to ACT. The **PSA** bound to alpha 2-M is caged by the inhibitor and thus escapes detection by antibodies. This results in an incorrect calculation of the level of **PSA** when released from prostate into the blood. Complexes of **PSA**-alpha 2-M and **PSA**-ACT were found to bind to the alpha 2-macroglobulin receptor/LDL receptor-related protein (alpha 2-M-R/LRP) which may be the clearance receptor for **PSA**. CONCLUSIONS: Quantifying free **PSA** and **PSA**-ACT complexes, as routinely done in managing prostate-associated diseases, does not represent the total...

... The proteinase inhibitor alpha 2-M has to be considered as a main contributor to **PSA** complex formation in the blood.

Descriptors: alpha-Macroglobulins--Metabolism--ME; ***Prostate-Specific Antigen***--Metabolism--ME; *Receptors, Immunologic --Metabolism--ME; *Receptors, LDL--Metabolism--ME

Enzyme No.: EC 3.4.21.77 (**Prostate-Specific Antigen**)

Chemical Name: **Prostate-Specific Antigen**; (alpha-Macroglobulins; (alpha-2-macroglobulin receptor; (Receptors, Immunologic; (Receptors, LDL

17/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

17/3/

09294160 98014582

Specific and efficient peptide substrates for assaying the proteolytic activity of **prostate-specific antigen**.

Denmeade SR; Lou W; Lovgren J; Malm J; Lilja H; Isaacs JT

The Johns Hopkins Oncology Center, Johns Hopkins School of Medicine, Baltimore, Maryland 21231-1001, USA.

Cancer research (UNITED STATES) Nov 1 1997, 57 (21) p4924-30, ISSN 0008-5472 Journal Code: CNF

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Prostate-specific antigen (**PSA**) is a serine protease secreted by both normal prostate glandular cells and prostate cancer cells. The major proteolytic substrates for **PSA** are the gel-forming proteins in semen, semenogelin (Sg) I and II. On the basis of the **PSA cleavage** map for Sg I and II, a series of small peptides (i.e., < or = 7 amino acids) was synthesized and coupled at the COOH terminus to 7-amino-4-methyl coumarin. Using these fluorescently tagged substrates, K(m)s and k(cat)s were determined for **PSA** hydrolysis, and the substrates were also tested for activity against a panel of purified proteases. Previously, a variety of chymotrypsin substrates have been used to assay the enzymatic activity of **PSA**. The present studies have identified a peptide sequence with a high degree of specificity for **PSA** (ie., no detectable hydrolysis by chymotrypsin) and improved K(m)s and k(cat)s over previously used substrates. On the basis of these parameters, the best peptide substrate for **PSA** has the amino acid sequence HSSKLQ. Using PC-82 human prostate cancer xenografts and human prostate tissues, this **PSA** substrate was used to document that prostate cancer cells secrete enzymatically active **PSA** into the extracellular fluid but that once in the blood, **PSA** is not enzymatically active. On the basis of this information, it should be possible to use the HSSKLQ peptide as a carrier to target peptide-coupled prodrugs for selective activation within **sites** of **PSA** -secreting, metastatic prostate cancer cells and not within the blood or other nonprostatic normal tissues.

Specific and efficient peptide substrates for assaying the proteolytic activity of **prostate-specific antigen**.

Prostate-specific antigen (**PSA**) is a serine protease secreted by both normal prostate glandular cells and prostate cancer cells. The major proteolytic substrates for **PSA** are the gel-forming proteins in semen, semenogelin (Sg) I and II. On the basis of the **PSA cleavage** map for Sg I and II, a series of small peptides (i.e., < or = 7...

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substrates. On the basis of these parameters, the best peptide substrate for **PSA** has the amino acid sequence HSSKLQ. Using PC-82 human prostate cancer xenografts and human prostate tissues, this **PSA** substrate was used to document that prostate cancer cells secrete enzymatically active **PSA** into the extracellular fluid but that once in the blood, **PSA** is not enzymatically active. On the basis of this information, it should be possible to...

... the HSSKLQ peptide as a carrier to target peptide-coupled prodrugs for selective activation within **sites** of **PSA**-secreting, metastatic prostate cancer cells and not within the blood or other nonprostatic normal tissues.

Descriptors: Peptide Fragments--Metabolism--ME; ***Prostate-Specific Antigen**--Metabolism--ME; *Prostatic Neoplasms --Metabolism--ME; *Sex Hormones--Metabolism--ME; alpha 1-Antichymotrypsin --Metabolism--ME; **Enzyme** Inhibitors--Metabolism--ME; Extracellular Space--Enzymology--EN; Hydrolysis; Kinetics; Mice; Mice, Nude; Molecular Sequence Data; Peptide Fragments--Chemistry--CH; Peptide Mapping; Precipitin Tests; Prodrugs--Metabolism--ME; **Prostate-Specific Antigen**--Blood--BL; Prostatic Neoplasms--Blood--BL; Substrate Specificity; Thapsigargin--Metabolism--ME; Transplantation, Heterologous; Tumor Cells...

Enzyme No.: EC 3.4.21.77 (**Prostate-Specific Antigen**)

Chemical Name: **Prostate-Specific Antigen**; (alpha 1-Antichymotrypsin; (semenogelin; (**Enzyme** Inhibitors; (Peptide Fragments; (Prodrugs; (Sex Hormones; (Thapsigargin

17/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09135285 97292068

Prostate specific antigen in benign prostatic hyperplasia: purification and characterization.

Chen Z; Chen H; Stamey TA

Department of Urology, Stanford University School of Medicine, California 94305-5118, USA.

Journal of urology (UNITED STATES) Jun 1997, 157 (6) p2166-70, ISSN 0022-5347 Journal Code: KC7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

PURPOSE: The ratio of free-to-total **prostate specific antigen** (**PSA**) in serum is greater in patients with benign prostatic hyperplasia (BPH) than in those with prostate cancer, and it provides a means of partially discriminating these 2 diseases. To understand the molecular mechanism of why the free-to-total **PSA** ratio is greater in BPH than in prostate cancer we analyzed **PSA** obtained directly from nodules of BPH tissue. MATERIALS AND METHODS: **PSA** from BPH nodule fluids was first purified by gel filtration and ion exchange chromatography. Purified BPH **PSA** was characterized by gel electrophoresis, **enzyme** assay and N-terminal sequence analysis of the amino acids. RESULTS: A single band at 30 kDa. on sodium dodecyl sulfate polyacrylamide gel electrophoresis under nonreducing conditions was identical to that of seminal fluid **PSA**. Under reducing conditions most BPH **PSA** was degraded, whereas most seminal fluid **PSA** existed as an intact molecule. BPH **PSA** had multiple internal **cleavages** in addition to the common **cleavage site** between lysines 145 and 146 of seminal fluid **PSA**. **Cleavage sites** at C-terminals of histidine 54 and phenylalanine 57 were also detected. Enzymatic activity studies with different substrates showed that **PSA** from seminal fluid and BPH nodules had similar specific trypsin-like activity. However, BPH **PSA** had much lower specific chymotrypsin-like activity than seminal fluid **PSA**. N-terminal sequence analysis showed that BPH **PSA** was neither in the pre-proenzyme form (261 amino acids)

nor the zymogen proenzyme form (244 amino acids) of **PSA**, both of which are known precursors of mature **PSA** (237 amino acids). CONCLUSIONS: Most **PSA** in BPH nodules is in the nicked form with low chymotrypsin-like activity. When it leaks into the circulation it will form fewer **PSA**-antichymotrypsin complexes and more will remain in the free form. We predict that a protease with trypsin-like activity in BPH nodule fluid is probably responsible for the nicked form of BPH **PSA**. These findings suggest that antibodies produced against **PSA** in BPH nodules may be useful in discriminating prostate cancer from BPH.

Prostate specific antigen in benign prostatic hyperplasia: purification and characterization.

PURPOSE: The ratio of free-to-total **prostate specific antigen** (**PSA**) in serum is greater in patients with benign prostatic hyperplasia (BPH) than in those with...

... discriminating these 2 diseases. To understand the molecular mechanism of why the free-to-total **PSA** ratio is greater in BPH than in prostate cancer we analyzed **PSA** obtained directly from nodules of BPH tissue. MATERIALS AND METHODS: **PSA** from BPH nodule fluids was first purified by gel filtration and ion exchange chromatography. Purified BPH **PSA** was characterized by gel electrophoresis, **enzyme** assay and N-terminal sequence analysis of the amino acids. RESULTS: A single band at ...

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...like activity in BPH nodule fluid is probably responsible for the nicked form of BPH **PSA** . These findings suggest that antibodies produced against **PSA** in BPH nodules may be useful in discriminating prostate cancer from BPH.

Descriptors: Prostate--Chemistry--CH; ***Prostate-Specific Antigen**--Analysis--AN; *Prostatic Hyperplasia--Metabolism--ME; **Prostate-Specific Antigen**--Metabolism--ME; Sequence Analysis

Enzyme No.: EC 3.4.21.77 (**Prostate-Specific Antigen**)

Chemical Name: **Prostate-Specific Antigen**

17/3,K,AB/7 (Item 7 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

7/31

08704312 96112170

Prostate-specific antigen activates single-chain urokinase-type plasminogen activator.

Yoshida E; Ohmura S; Sugiki M; Maruyama M; Mihara H

Department of Physiology, Miyazaki Medical College, Japan.

International journal of cancer. Journal international du cancer (UNITED

STATES) Dec 11 1995, 63 (6) p863-5, ISSN 0020-7136 Journal Code: GQU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Prostate-specific antigen (**PSA**) increases in the plasma of patients with prostate cancer, and has therefore been used as a reliable tumor marker. It has been demonstrated that prostate cancer cells over-express urokinase-type plasminogen activator (uPA), which plays an important role in tumor invasion and metastasis. We found that **PSA** converts the single-chain proform of urokinase-type plasminogen activator (scuPA) to an active 2-chain form. The active 2-chain uPA generated from scuPA by **PSA** was measured by hydrolyzation of S-2444, a synthetic substrate for uPA. **PSA** activated scuPA time- and dose-dependently. SDS-PAGE analysis revealed that, after incubation with **PSA**, the intensity of the 55-kDa band of scuPA decreased concomitantly with increases in the intensity of the 2 bands at 33 kDa and 22 kDa. Amino-acid-sequence analysis indicated that **PSA cleaved** Lys158-Ile159, which corresponds with the **site cleaved** by plasmin. **PSA** did not enhance or impair the activity of the 2-chain form of uPA. These findings imply that **PSA** could be an initiator of the protease cascade involved in prostate-cancer invasion and metastasis.

Prostate-specific antigen activates single-chain urokinase-type plasminogen activator.
Prostate-specific antigen (**PSA**) increases in the plasma of patients with prostate cancer, and has therefore been used as...

... activator (uPA), which plays an important role in tumor invasion and metastasis. We found that **PSA** converts the single-chain proform of urokinase-type plasminogen activator (scuPA) to an active 2-chain form. The active 2-chain uPA generated from scuPA by **PSA** was measured by hydrolyzation of S-2444, a synthetic substrate for uPA. **PSA** activated scuPA time- and dose-dependently. SDS-PAGE analysis revealed that, after incubation with **PSA** , the intensity of the 55-kDa band of scuPA decreased concomitantly with increases in the...

...the 2 bands at 33 kDa and 22 kDa. Amino-acid-sequence analysis indicated that **PSA cleaved** Lys158-Ile159, which corresponds with the **site cleaved** by plasmin. **PSA** did not enhance or impair the activity of the 2-chain form of uPA. These findings imply that **PSA** could be an initiator of the protease cascade involved in prostate-cancer invasion and metastasis.
Descriptors: **Prostate-Specific Antigen**--Metabolism--ME; *Urinary Plasminogen Activator--Metabolism--ME; Amino Acid Sequence; **Enzyme** Activation; Molecular Sequence Data
Enzyme No.: EC 3.4.21.73 (Urinary Plasminogen Activator); EC 3.4.21.77 (**Prostate-Specific Antigen**)
Chemical Name: Urinary Plasminogen Activator; (**Prostate-Specific Antigen**

17/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08428123 96062531
Purification and characterization of different molecular forms of **prostate-specific antigen** in human seminal fluid.
Zhang WM; Leinonen J; Kalkkinen N; Dowell B; Stenman UH
Department of Clinical Chemistry, Helsinki University Central Hospital, Finland.
Clinical chemistry (UNITED STATES) Nov 1995, 41 (11) p1567-73, ISSN 0009-9147 Journal Code: DBZ
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have developed a new procedure for purifying **prostate-**

specific antigen (**PSA**) from human seminal fluid. The method is based on ammonium sulfate precipitation, hydrophobic interaction chromatography, gel filtration, and anion-exchange chromatography. It can be completed within 2 days with a recovery of intact **PSA** of 30%. By anion-exchange chromatography, five isoforms of **PSA** (A, B, C, D, and E) can be separated. The major form (**PSA**-B) consists of the intact **enzyme** , as shown by the occurrence of only one band of 33 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing or nonreducing conditions, and by amino acid sequencing, which reveals only one amino-terminal sequence corresponding to the reported amino-terminal sequence of intact **PSA**. The specific absorbance of 1 g/L **PSA**-B at 280 nm was 1.61, and 80% of the **PSA**-B formed a complex with alpha 1-antichymotrypsin, indicating that it is enzymatically active. Three **cleaved** forms of **PSA** with different nicking **sites** and low enzymatic activity were separated from intact **PSA** by ion-exchange chromatography. In addition, we isolated a glycosylation variant, **PSA** -A, which showed a higher isoelectric point (pI = 7.2) than **PSA**-B (pI = 6.9) but similar enzymatic activity; this form accounts for 5-10% of total **PSA**. After treatment with sialidase, **PSA**-A and B had the same isoelectric point value (pI = 7.7).

Purification and characterization of different molecular forms of **prostate-specific antigen** in human seminal fluid.
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Descriptors: **Prostate-Specific Antigen**--Isolation and Purification--IP; *Semen--Immunology--IM...; Electrophoresis, Polyacrylamide Gel; Isoelectric Focusing; Molecular Sequence Data; Molecular Weight; Peptide Fragments--Chemistry--CH; Precipitation; **Prostate-Specific Antigen**--Chemistry--CH; **Prostate-Specific Antigen**--Metabolism--ME; Sequence Analysis
Enzyme No.: EC 3.4.21.77 (**Prostate-Specific Antigen**)
Chemical Name: **Prostate-Specific Antigen**; (alpha 1-Antichymotrypsin; (Peptide Fragments; (Ammonium Sulfate

17/3,K,AB/9 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

12043751 BIOSIS NO.: 199900324270
Enzymatic action of human glandular kallikrein 2 (hK2): Substrate specificity and regulation by Zn2+ and extracellular protease inhibitors.
AUTHOR: Lovgren Janita(a); Airas Kalervo; Lilja Hans
AUTHOR ADDRESS: (a)Department of Biotechnology, University of Turku,

Tykistokatu 6, 20520, Turku**Finland
1999
JOURNAL: European Journal of Biochemistry 262 (3):p781-789 June, 1999
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Human glandular kallikrein 2 (hK2) is a serine protease expressed by the prostate gland with 80% identity in primary structure to **prostate-specific antigen** (**PSA**). Recently, hK2 was shown to activate the zymogen form of **PSA** (proPSA) in vitro and is likely to be the physiological activator of **PSA** in the prostate. hK2 is also able to activate urokinase and effectively **cleave** fibronectin. We studied the substrate specificity of hK2 and regulation of its activity by zinc and extracellular protease inhibitors present in the prostate and seminal plasma. The enzymatic activity and substrate specificity was studied by determining hK2 **cleavage sites** in the major gel proteins in semen, semenogelin I and II, and by measuring hydrolysis of various tripeptide aminomethylcoumarin substrates. HK2 **cleaves** substrates C-terminal of single or double arginines. Basic amino acids were also occasionally found at several other positions N-terminal of the **cleavage site**. Therefore, the substrate specificity ofhK2 fits in well with that of a processor of protein precursors. Possible regulation mechanisms were studied by testing the ability of Zn2+ and different protease inhibitors to inhibit hK2 by kinetic measurements. Inhibitory constants were determined for the most effective inhibitors PCI and Zn2+. The high affinity of PCI for hK2 (kass = 2.0 X 105 M-1cntdots-1) and the high concentrations of PCI (4 muM) and hK2 (0.2 muM) in seminal plasma make hK2 a very likely physiological target protease for PCI. hK2 is inhibited by Zn2+ at micromolar concentrations well below the 9 mM zinc concentration found in the prostate. The enzymatic activity of hK2 is likely to be reversibly regulated by Zn2+ in prostatic fluid. This regulation may be impaired in CAP and advanced metastatic cancer resulting in lack of control of the hK2 activity and a need for other means of control.
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DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...**prostate-specific antigen**;
MISCELLANEOUS TERMS: **enzyme** activity

17/3,K,AB/10 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07662351 Genuine Article#: 193NM Number of References: 80

Title: Combinatorial strategies for the discovery of novel protease specificities
Author(s): Lien S; Francis GL; Graham LD (REPRINT)
Corporate Source: CSIRO,SYDNEY LAB, POB 184/N RYDE/NSW 2113/AUSTRALIA/ (REPRINT); CSIRO,SYDNEY LAB/N RYDE/NSW 2113/AUSTRALIA/; COOPERAT RES CTR TISSUE GROWTH & REPAIR,/ADELAIDE/SA 5000/AUSTRALIA/
Journal: COMBINATORIAL CHEMISTRY & HIGH THROUGHPUT SCREENING, 1999, V2, N2 (APR), P73-90
ISSN: 1386-2073 Publication date: 19990400
Publisher: BENTHAM SCIENCE PUBL BV, PO BOX 1673, 1200 BR HILVERSUM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: This article discusses proven and possible ways to generate novel **cleavage** specificities in serine proteases using combinatorial mutagenesis, compares the different ways of screening or selecting for desirable mutants, and examines the ways in which combinatorial substrate libraries can be used to gain a more comprehensive insight into protease **cleavage** preferences. The use of bacteriophage to display both combinatorial protease libraries and combinatorial substrate libraries will be discussed.
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...Identifiers--ALPHA-LYTIC PROTEASE; INTERLEUKIN-1-BETA CONVERTING-**ENZYME; PROSTATE-SPECIFIC ANTIGEN;**
SUBSTRATE-BINDING **SITE**; PHAGE DISPLAY; RANDOM MUTAGENESIS; PLASMINOGEN-ACTIVATOR; SERINE PROTEASES; IN-VIVO; RAPID IDENTIFICATION

17/3,K,AB/11 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06294295 Genuine Article#: YG647 Number of References: 44
Title: Serpin-derived peptide substrates for investigating the substrate specificity of human tissue kallikreins hK1 and hK2
Author(s): Bourgeois L; BrillardBourdet M; Deperthes D; Juliano MA; Juliano L; Tremblay RR; Dube JY; Gauthier F (REPRINT)
Corporate Source: UNIV TOURS,ENZYMOL & PROT CHEM LAB, CNRS, EP 117, 2BIS BD TONNELLE/F-37032 TOURS//FRANCE/ (REPRINT); UNIV TOURS,ENZYMOL & PROT CHEM LAB, CNRS, EP 117/F-37032 TOURS//FRANCE/; CHUL,RES CTR, HORMONAL BIOREGULAT LAB/ST FOY/PQ/CANADA/; UNIV FED SAO PAULO,ESCOLA PAULISTA MED, DEPT BIOPHYS/SAO PAULO//BRAZIL/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1997, V272, N47 (NOV 21), P 29590-29595
ISSN: 0021-9258 Publication date: 19971121
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Abstract: The third human tissue kallikrein to be identified, hK2, could be an alternate or complementary marker to kallikrein hK3 (**prostate-specific antigen**) for prostate diseases, Most of the hK2 in seminal plasma forms an inactive complex with protein C inhibitor (PCI), a serpin secreted by seminal vesicles, As serpin inhibitors behave as suicide substrates that are **cleaved** early in the interaction with their target **enzyme**, and kallikreins have different sensitivities to serpin inhibitors, we prepared a series of substrates with intramolecularly quenched fluorescence based on the sequences of the serpin reactive loops, They were used to compare the substrate specificities of hK1 and hK2, which both have trypsin-like

specificity, and thus differ from chymotrypsin-like hK3, The serpin-derived peptides behaved as kallikrein substrates whose sensitivities reflected the specificity of the parent inhibitory proteins, Substrates derived from PCI were the most sensitive for both hK1 and hK2 with specificity constants of about 10(7) M-1.s(-1). Those derived from antithrombin III and alpha(2)-antiplasmin were more specific for hK2 while a kallistatin-derived substrate was specifically **cleaved** by hK1, hK1 and hK2 substrates of greater specificity were obtained using chimeric peptides based on the sequence of serpin reactive loops,

The main difference between specificities of hK1 and hK2 arise because hK2 can accommodate positively charged as well as small residues at P-2 and requires an arginyl residue at P-1, Thus, unlike hK1, hK2 does not **cleave** kininogen derived substrates overlapping the region of N-terminal insertion of bradykinin in human kininogens.

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...Identifiers--**PROSTATE-SPECIFIC ANTIGEN**; HUMAN GLANDULAR KALLIKREIN; PROTEIN-C INHIBITOR; RAT-SUBMAXILLARY-GLAND; SUICIDE SUBSTRATE; SEMINAL PLASMA; HUMAN SEMEN; PURIFICATION; EXPRESSION; **SITE**

Research Fronts: 95-0297 001 (**PROSTATE-SPECIFIC ANTIGEN** ; EARLY DETECTION; SCREENING IN GENERAL-PRACTICE)
95-2633 001 (NONPEPTIDE BRADYKININ B-2 RECEPTOR ANTAGONISTS...

17/3,K,AB/12 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05965207 Genuine Article#: XK776 Number of References: 40
Title: The crystal structure of the mouse glandular kallikrein-13 (prorenin converting **enzyme**)
Author(s): Timm DE (REPRINT)
Corporate Source: OAK RIDGE NATL LAB,DIV BIOL, POB 2009, MS8077/OAK RIDGE//TN/37831 (REPRINT); UNIV LONDON BIRKBECK COLL,DEPT CRYSTALLOG, ICRF UNIT/LONDON WC1E 7HX//ENGLAND/
Journal: PROTEIN SCIENCE, 1997, V6, N7 (JUL), P1418-1425
ISSN: 0961-8368 Publication date: 19970700
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211
Language: English Document Type: ARTICLE
Abstract: A crystal structure of the serine protease, mouse glandular kallikrein 13 (mGK-13) has been determined at 2,6-A resolution. This **enzyme**, isolated from the mouse submandibular gland, is also known as prorenin-converting **enzyme** and **cleaves** submandibular gland Ren-2 prorenin to yield active renin. The mGK-13 structure is similar to other members of the mammalian serine protease

family, having five conserved disulfide bonds and an active **site** located in the cleft between two beta-barrel domains. The mGK-13 structure reveals for the first time an ordered kallikrein loop conformation containing a short 3(10) helix. This loop is disordered in the related porcine pancreatic kallikrein and rat submandibular tonin structures. The kallikrein loop is in close spatial proximity to the active **site** and is also involved in a dimeric arrangement of mGK-13. The catalytic specificity of mGK-13 for Ren-2 prorenin was studied by modeling a prorenin-derived peptide into the active **site** of mGK-13. This model emphasizes two electronegative substrate specificity pockets on the mGK-13 surface, which could accommodate the dibasic P2. and Pt residues at the sits of prorenin **cleavage** by mGK-13.

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...Identifiers--EPIDERMAL GROWTH-FACTOR; FACTOR-BINDING-PROTEIN; **PROSTATE-SPECIFIC ANTIGEN**; SUBMANDIBULAR-GLAND; SYNTHETIC CHIMERAS; ALPHA-CHYMOTRYPSIN; SUBMAXILLARY-GLAND; BOVINE TRYPSIN; RESOLUTION; REFINEMENT

17/3,K,AB/13 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05208926 Genuine Article#: VH272 Number of References: 31
Title: POLYSIALYLATION OF NCAM BY A SINGLE **ENZYME**
Author(s): MUHLENHOFF M; ECKHARDT M; BETHE A; FROSCH M; GERARDYSCHAHN R
Corporate Source: HANNOVER MED SCH,INST MED MIKROBIOL,KONSTANTY GUTSHCOW STR 8/D-30625 HANNOVER//GERMANY/; HANNOVER MED SCH,INST MED MIKROBIOL/D-30625 HANNOVER//GERMANY/
Journal: CURRENT BIOLOGY, 1996, V6, N9 (SEP 1), P1188-1191
ISSN: 0960-9822
Language: ENGLISH Document Type: ARTICLE
Abstract: The addition of poly-alpha 2,8-N-acetylneuraminic acid (polysialic acid; **PSA**) to the neural cell adhesion molecule NCAM plays a crucial role in neural development [1-3], neural regeneration [4], and plastic processes in the vertebrate brain associated with neurite outgrowth [5], axonal pathfinding [6], and learning and memory [7-9]. **PSA** levels are decreased in people affected by schizophrenia [10], and **PSA** has been identified as a specific marker for some neuroendocrine and lymphoblastoid tumours [11-13]; expression of **PSA** on the surface of these tumour cells modulates their metastatic potential [11,13]. Studies aimed at understanding **PSA** biosynthesis and the dynamics of its production have largely been promoted by the cloning of polysialyltransferases (PST-1 in

hamster; PST in human and mouse) [14-16]. However, the number of enzymes involved in the biosynthesis of **PSA** has not been identified. Using incompletely glycosylated NCAM variants and soluble recombinant glycosyltransferases, we reconstituted the **site** at which PST-1 acts to polysialylate NCAM in vitro. The data presented here clearly demonstrate that polysialylation of NCAM is catalyzed by a single **enzyme**, PST-1, and that terminal sialylation of the N glycan core is sufficient to generate the **PSA** acceptor **site** . Our results also show that PST-1 can act on core structures with the terminal sialic acid connected to galactose via an alpha 2,3 or alpha 2,6 linkage.

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...Identifiers--CELL-ADHESION MOLECULE; POLYSIALIC ACID; EXPRESSION; CLONING; SURFACE; BRAIN; INVOLVEMENT; **CLEAVAGE**; NEURONS; LUNG

17/3,K,AB/14 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04733934 Genuine Article#: UD850 Number of References: 24
Title: PHASE-II STUDY OF TOPOTECAN IN METASTATIC HORMONE-REFRACTORY PROSTATE-CANCER
Author(s): HUDES GR; KOSIEROWSKI R; GREENBERG R; RAMSEY HE; FOX SC; OZOLS RF; MCALEER CA; GIANTONIO BJ
Corporate Source: FOX CHASE CANC CTR,DEPT MED ONCOL,ROOM W250,7701 BURHOLME AVE/PHILADELPHIA//PA/19111; FOX CHASE CANC CTR,DEPT SURG ONCOL/PHILADELPHIA//PA/19111; FOX CHASE NETWORK/PHILADELPHIA//PA/00000
Journal: INVESTIGATIONAL NEW DRUGS, 1995, V13, N3, P235-240
ISSN: 0167-6997
Language: ENGLISH Document Type: ARTICLE
Abstract: Systemic chemotherapy with currently available agents has not improved survival for patients with hormone refractory prostate cancer (HRPC), consequently, the evaluation of new agents is warranted. Topotecan is a specific inhibitor of topoisomerase I with broad antitumor activity in preclinical studies. The purpose of this phase II trial was to determine the objective response rate of topotecan administered as a 30 minute infusion for five consecutive days in men with metastatic HRPC. Thirty-four evaluable patients were treated with topotecan 1.1-1.5 mg/m(2) as a 30 minute infusion daily for five days, repeated every three weeks until disease progression or unacceptable

toxicity. Response was assessed with a combination of standard solid tumor response criteria and the serum **prostate specific antigen** (**PSA**) for patients with bidimensionally measurable disease, and by serial measurements of the **PSA** in patients with bone only (evaluable) disease. One of 13 patients (7.6%) with measurable soft tissue disease had a PR in nodal **sites**. Of 21 patients with only osseous metastases, 1 (4.7%) had improvement in bone scan. Six of the 34 evaluable patients (17.6%) had the serum **PSA** decrease by greater than or equal to 50% and 2 (5.8%) had **PSA** decreases of greater than or equal to 75%. Toxicity was chiefly hematologic with 66% of patients experiencing Grade 3 or 4 granulocytopenia. Thirty-nine percent of cycles required a delay to allow for hematologic recovery and ten patients required red cell transfusions. Nonhematologic toxicity, mainly nausea and alopecia, was mild. Topotecan administered at this dose and schedule has limited activity in patients with HRPC. Further trials of topo I inibition in HRPC should utilize alternative schedules of topotecan (e.g., prolonged infusion) or other camptothecin analogs with more potent topo I inhibitory activity.

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...Identifiers--DNA UNTWISTING **ENZYME**; TOPOISOMERASE-I; SOLID TUMORS; CAMPTOTHECIN; INHIBITOR; PAIN
...Research Fronts: CELL LUNG-CANCER; CHEMOTHERAPY PLUS RADICAL RADIOTHERAPY; SEQUENCE SPECIFICITY OF DRUG-STIMULATED TOPOISOMERASE-II DNA **CLEAVAGE**)
94-4198 001 (SURAMIN IN-VITRO; BASIC FIBROBLAST GROWTH-FACTOR; CENTRAL VASCULAR SMOOTH-MUSCLE CELLS)

17/3,K,AB/15 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03559835 Genuine Article#: PK877 Number of References: 28
Title: HUMAN FIBROBLASTS SECRETE A SERINE-PROTEASE THAT **CLEAVES** INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-5
Author(s): NAM TJ; BUSBY WH; CLEMMONS DR
Corporate Source: UNIV N CAROLINA,SCH MED,DEPT MED,DIV ENDOCRINOL,CB 7170/CHAPEL HILL//NC/27599; UNIV N CAROLINA,SCH MED,DEPT MED,DIV ENDOCRINOL/CHAPEL HILL//NC/27599
Journal: ENDOCRINOLOGY, 1994, V135, N4 (OCT), P1385-1391
ISSN: 0013-7227
Language: ENGLISH Document Type: ARTICLE
Abstract: We have previously reported the presence of proteolytic activity in conditioned medium from human fibroblast cultures that **cleaves** insulin-like growth factor-binding protein-5 (IGFBP-5) into non-IGF-I-binding fragments. Coincubation of IGF-I or IGF-II and IGFBP-5 with fibroblast cultures decreased proteolysis. The protease was purified by heparin-Sepharose affinity chromatography. The purified protease **cleaved** IGFBP-5 into 22-, 20-, and 17-kilodalton non-IGF-I-binding fragments. Protease inhibitor profiles obtained using partially purified **enzyme** showed that it was a calcium-dependent serine protease. After chelation with EDTA, the activity could only be partially restored with zinc, indicating that it was probably not a

metalloprotease. The protease was specific for IGFBP-5 and did not **cleave** pure IGFBP-1, -2, -3, or -4. IGF-I and IGF-II caused minimal inhibition of proteolysis in. vitro. This suggests that the IGF-I-induced increase in IGFBP-5 in fibroblast medium is only partially due to direct protease inhibition. Heparin, antithrombin-III (AT-III), and heparin cofactor-II had inhibitory activity, and heparin potentiated the activity of AT-III. Synthetic peptides, that contained the active **sites** of AT-III and alpha(1)-antichymotrypsin, were also inhibitory. Peptides containing sequences found in two basic regions of IGFBP-5 were tested, and one had inhibitory activity. In summary, fibroblasts secrete a serine protease that **cleaves** IGFBP-5 and is specific for this form of IGFBP. The protease has properties that are similar to kallikreins, a family of serine proteases that is known to **cleave** epidermal and nerve growth factor-binding proteins.

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...Identifiers--CULTURED HUMAN-FIBROBLASTS; **PROSTATE-SPECIFIC ANTIGEN**; HEPARIN COFACTOR; MESSENGER-RNA; MUSCLE-CELLS; GENE FAMILY; IGF-BINDING; 2 FORMS; PURIFICATION; SIMILARITY

17/3,K,AB/16 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02714279 Genuine Article#: LX852 Number of References: 41
Title: POSTTRANSLATIONAL MODIFICATIONS OF THE DICTYOSTELIUM-DISCOIDEUM GLYCOPROTEIN **PSA** - GLYCOSYLPHOSPHATIDYLINOSITOL MEMBRANE ANCHOR AND COMPOSITION OF O-LINKED OLIGOSACCHARIDES
Author(s): HAYNES PA; GOOLEY AA; FERGUSON MAJ; REDMOND JW; WILLIAMS KL
Corporate Source: MACQUARIE UNIV,SCH BIOL SCI,CTR ANALYT BIOTECHNOL/N RYDE/NSW 2109/AUSTRALIA/; MACQUARIE UNIV,SCH BIOL SCI,CTR ANALYT BIOTECHNOL/N RYDE/NSW 2109/AUSTRALIA/; UNIV DUNDEE,DEPT BIOCHEM/DUNDEE DD1 4HN//SCOTLAND/
Journal: EUROPEAN JOURNAL OF BIOCHEMISTRY, 1993, V216, N3 (SEP 15), P 729-737
ISSN: 0014-2956
Language: ENGLISH Document Type: ARTICLE

Abstract: Prespore-specific antigen (**PsA**) is a cell-surface glycoprotein isolated from Dictyostelium discoideum, which is post-translationally modified by addition of carbohydrate to threonine residues of the carboxy-terminal peptide domain, and a glycosylphosphatidylinositol (GPI) anchor which attaches the glycoprotein to the cell membrane. The GPI anchor was isolated by proteolytic **cleavage** of the protein, and the structure of the lipid and glycan portions of the anchor were determined. The lipid moiety of the anchor is an inositolphosphoceramide which contains C18:0 phytosphingosine as a long chain base, and a mixture of fatty acids with a Cl 8: 1 mono-unsaturated fatty acid as the major component. The purified GPI anchor was susceptible to digestion by a bacterial phosphatidylinositol-specific phospholipase-C **enzyme**. The glycan of the GPI anchor consisted of two molecular species present in the ratio 55:45, the structures of which were determined by exoglycosidase sequencing and found to be Manalpha1-2Manalpha1-6Manalpha1-4GlcNH2 and Manalpha1-2Manalpha1-2Manalpha1-6Manalpha1-4GlcNH2. The glucosamine in both structures is glycosidically linked to the inositol ring of the inositolphosphoceramide. The GPI glycan structures are consistent with the conserved core structure of all characterised GPI anchors, and the structure of the D. discoideum GPI moiety has features in common with structures from yeast, protozoa and higher eukaryotes.

Compositional analysis of the carbohydrate attached to threonine residues in the carboxy-terminal peptide domain is also presented. The oligosaccharides bind to wheat germ agglutinin, and contain glucosamine and fucose as the major constituents.

Title: POSTTRANSLATIONAL MODIFICATIONS OF THE DICTYOSTELIUM-DISCOIDEUM GLYCOPROTEIN **PSA** - GLYCOSYLPHOSPHATIDYLINOSITOL MEMBRANE ANCHOR AND COMPOSITION OF O-LINKED OLIGOSACCHARIDES
Abstract: Prespore-specific antigen (**PsA**) is a cell-surface glycoprotein isolated from Dictyostelium discoideum, which is post-translationally modified by...

...which attaches the glycoprotein to the cell membrane. The GPI anchor was isolated by proteolytic **cleavage** of the protein, and the structure of the lipid and glycan portions of the anchor...

...The purified GPI anchor was susceptible to digestion by a bacterial phosphatidylinositol-specific phospholipase-C **enzyme**. The glycan of the GPI anchor consisted of two molecular species present in the ratio...
...Identifiers--HUMAN-ERYTHROCYTE ACETYLCHOLINESTERASE; CELL-SURFACE GLYCOPROTEIN; **SITE**-A GLYCOPROTEIN; STRUCTURAL CHARACTERIZATION; PHOSPHOLIPID ANCHOR; TRYPANOSOMA-CRUZI; LEISHMANIA-MAJOR; PROTEINS; ACID; IDENTIFICATION

17/3,K,AB/17 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01698904 Genuine Article#: HT965 Number of References: 44
Title: PROTEIN PRODUCTS OF THE RAT KALLIKREIN GENE FAMILY - SUBSTRATE SPECIFICITIES OF KALLIKREIN RK2 (TONIN) AND KALLIKREIN RK9
Author(s): MOREAU T; BRILLARDBOURDET M; BOUHNIK J; GAUTHIER F
Corporate Source: UNIV TOURS,FAC MED,CNRS,URA 1334,ENZYMOL & CHIMPROT LAB,2BIS,BD TONNELLE/F-37032 TOURS//FRANCE/; UNIV TOURS,FAC MED,CNRS,URA 1334,ENZYMOL & CHIMPROT LAB,2BIS,BD TONNELLE/F-37032 TOURS//FRANCE/; INSERM,U36/F-75005 PARIS//FRANCE/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1992, V267, N14 (MAY 15), P 10045-10051
Language: ENGLISH Document Type: ARTICLE
Abstract: Two closely related kallikrein-like proteinases having little

activity toward the standard synthetic amide substrates of tissue kallikreins were isolated from the rat submandibular gland. They were found to be the protein products of the rKlk2 (tonin) and the rKlk9 genes by amino acid sequence analysis (nomenclature of the genes and proteins of the kallikrein family is according to the proposal of the discussion panel from the participants of the KININ '91 meeting held Sept. 8-14, 1991, in Munich, Germany). These two proteinases of similar structure also had very similar physicochemical properties. They differed from other kallikrein-related proteinases in having high pH(i) values of 6.20 (rK2) and 6.85 (rK9). Kallikrein rK2 was purified as a single peptide chain, whereas rK9 appeared as a two-chain protein after reduction. Their enzymatic properties were also very similar and differed significantly from those of other rat kallikrein-related proteinases. Unlike the five other kallikrein-related proteinases we have purified so far, kallikrein rK9 was not inhibited by aprotinin. rK9 also differed from rK2 by its tissue localization. The prostate gland contained only rK9 where it was the major kallikrein-like component.

The amino acids preferentially accommodated by the proteinase S3 to S2' subsites were identified using synthetic amide and protein substrates. Unlike other kallikrein-related proteinases, rK2 had a prevalent chymotrypsin-like specificity, whereas rK9 had both chymotrypsin-like and trypsin-like properties. Both rK2 and rK9 preferred a prolyl residue in position P2 of the substrate and did not accommodate bulky and hydrophobic residues at that position, as did most of the other kallikrein-related proteinases. This P2-proline-directed specificity is necessary for processing the precursors of several biologically active peptides. Subsites accommodating residues COOH-terminal to the scissile bond were also important in determining the overall substrate specificity of these proteinases. rK2 and rK9 both showed a preference for hydrophobic residues in P2'. Other subsites upstream of the S3 subsite were found to intervene in substrate binding and hydrolysis. The restricted specificity of rK2 and rK9 is consistent with the presence of an extended substrate binding **site**, and hence with a processing **enzyme** function. Their P1 specificities enabled both proteinases to release angiotensin II from angiotensinogen and from angiotensinogen I, but rK9 was at least 100 times less active than rK2 on both substrates. The substrate specificities of rK2 and rK9 were correlated with key amino acids defining their substrate binding **site**. The predicted preferential sequence(s) around the **cleavage site** deduced from these data may be used to identify the biological substrate(s) of these proteinases.

...Abstract: specificity of rK2 and rK9 is consistent with the presence of an extended substrate binding **site**, and hence with a processing **enzyme** function. Their P1 specificities enabled both proteinases to release angiotensin II from angiotensinogen and from...

...specificities of rK2 and rK9 were correlated with key amino acids defining their substrate binding **site**. The predicted preferential sequence(s) around the **cleavage site** deduced from these data may be used to identify the biological substrate(s) of these...

...Identifiers--**PROSTATE-SPECIFIC ANTIGEN**; AMINO-ACID-SEQUENCE; SUBMAXILLARY-GLAND; SERINE-PROTEASE; MESSENGER-RNA; CDNA CLONING; T-KININOGEN; PURIFICATION; **CLEAVAGE**; CONFIRMATION

17/3,K,AB/18 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3343326 IFI Acc No: 0019487

Document Type: C
NUCLEIC ACID CONSTRUCT FOR EXPRESSING ACTIVE SUBSTANCES WHICH CAN BE ACTIVATED BY PROTEASES, AND PREPARATION AND USE
Inventors: Heidtmann Hans Heinrich (DE); Mueller Rolf (DE); Sedlacek Hans-Harald (DE)
Assignee: Hoechst AG AG DE
Patent (No,Date), Applic (No,Date)
US 6080575 20000627 US 988308 19980116
Priority Applic(No,Date): DE 19701141 19970116

Abstract:
The invention relates to a nucleic acid construct for expressing an active substance which is activated by an **enzyme** which is released from mammalian cells, which construct comprises the following components: a) at least one promoter element, b) at least one DNA sequence which encodes an active compound (protein B) c) a least one DNA sequence which encodes an amino acid sequence (part structure C) which can be **cleaved** specifically by an **enzyme** which is released from a mammalian cell, and d) at least one DNA sequence which encodes a peptide or protein (part structure D) which is bound to the active compound (protein B) by way of the **cleavable** amino acid sequence (part structure C) and inhibits the activity of the active compound (protein B), and also to the use of the nucleic acid construct for preparing a drug for treating diseases.

Abstract:
...to a nucleic acid construct for expressing an active substance which is activated by an **enzyme** which is released from mammalian cells, which construct comprises the following components: a) at least...

...one DNA sequence which encodes an amino acid sequence (part structure C) which can be **cleaved** specifically by an **enzyme** which is released from a mammalian cell, and d) at least one DNA sequence which...

...structure D) which is bound to the active compound (protein B) by way of the **cleavable** amino acid sequence (part structure C) and inhibits the activity of the active compound (protein...

Exemplary Claim:
...isolated nucleic acid construct for expressing a biologically active substance which is activated by an **enzyme** which is released from mammalian cells, wherein said construct comprises the following nucleic acid sequences...

...linked to; c) at least one nucleic acid sequence which encodes an amino acid sequence **cleavable** specifically by a protease which is released from a mammalian cell, operably linked to; d...

...DNA sequence which encodes a polypeptide which is bound to said active compound by said **cleavable** amino acid sequence and inhibits the activity of said active compound, and wherein said nucleic...

Non-exemplary Claims:
2. A nucleic acid construct as claimed in claim 1, wherein said protease is a **prostate specific antigen**, a plasminogen activator, a cathepsin or a matrix metalloproteinase...

...natural precursor of a biologically active protein compound, wherein the nucleic acid sequence encoding the **cleavage** sequence naturally occurring between said nucleic acid sequences b) and d) has been replaced by...

...system, activates fibrinolysis, activates the complement system or activates the kinin system, or is an **enzyme** which converts the inactive precursor of a pharmacological substance into the pharmacologically active substance, or...

...fragments and factor XIIa; thrombin which is mutated in the region of the Arg-Thr **cleavage site** at amino acid position 327/328; a fibrinolytic protein selected from the group consisting of of CVF, C3b and functional **cleavage** products thereof; an antithrombotic protein selected from the group consisting of protein C, C-1S...

...nucleic acid sequences are linked to each other by way of an internal ribosomal entry **site**.

29/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06321041 88131253

Detection of alpha 2-macroglobulin, alpha 1-protease inhibitor, and neutral protease-antiprotease complexes within liver granulomas of Schistosoma mansoni-infected mice.

Truden JL; Boros DL

Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI 48201.

American journal of pathology (UNITED STATES) Feb 1988, 130 (2) p281-8
, ISSN 0002-9440 Journal Code: 3RS

Contract/Grant No.: AI-12913, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In schistosomiasis mansoni the parasite egg-induced granulomatous tissue inflammations resolve by fibrosis. Intralesional collagen synthesis and deposition are influenced by collagenase, elastase activity that is diminished at the chronic stage of the disease. To determine the cause of diminished neutral protease activity, the authors determined levels of the antiprotease/alpha 2-macroglobulin (alpha 2M) and alpha 1-protease inhibitor (alpha 1Pi) in extracts or secretions of liver granulomas of infected mice. By ELISA, both antiproteases were detected in granuloma-derived substances, as well as supernatants of cultured, adherent granuloma macrophages. In all samples, alpha 2M was the predominant inhibitor. Antiprotease levels were similar in granuloma-derived samples obtained from acutely and chronically infected mice. However, supernatants of cultured adherent macrophages isolated from granulomas of mice with acute infection contained levels of protease inhibitors several times higher than those of similar preparations obtained from chronically infected animals. Gel filtration of samples on Sephacryl S-200 columns did not separate collagenase and elastase from protease inhibitors. By chromatofocusing, a few inhibitor-free collagenase as well as enzyme-free alpha 2M and alpha 1Pi-active peaks were eluted. The bulk of the material that eluted at the acidic region contained protease-antiprotease activity indicating the presence of enzyme-inhibitor complexes. The intragranulomatous presence of antiproteases complexed with protease enzymes emphasizes their importance in the possible enhancement of fibrosis.

36/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05219592 86180527
Consumptive coagulopathy, fibrinolysis and protease-antiprotease interactions during acute human pancreatitis.
Lasson A; Ohlsson K
Thrombosis research (UNITED STATES) Jan 15 1986, 41 (2) p167-83,
ISSN 0049-3848 Journal Code: VRN
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Twenty-seven attacks of acute human pancreatitis of different severity were analysed concerning clinical outcome and activation of the coagulation and fibrinolytic systems. Consumptive coagulopathy was suggested by decreased platelet counts, decreased prothrombin values and consumption of fibrinogen during the first days in severe attacks. Factor X was slightly decreased the first 5 days in all attacks. Increased fibrinolysis was suggested by decreased **plasminogen** values in severe attacks. Fibrinogen degradation products were seen in 40% of the patients in **blood** and in 100% of the patients in the peritoneal fluid. The four main **protease inhibitors** of the two systems all showed **protease**-antiprotease **complexation** and lower functional than quantitative values. Plasma levels of antithrombin III and alpha 2-macroglobulin were low, while the levels of C1-inhibitor and alpha 2-antiplasmin were high. Functional levels of all the four protease inhibitors were almost zero in the peritoneal fluid in severe attacks. It is concluded that severe acute pancreatitis results in both consumptive coagulopathy and in increased fibrinolysis. A local antiprotease deficiency is seen in the peritoneal cavity and high levels of protease-antiprotease complexes are also seen in plasma. All these changes are closely correlated to the severity of the disease and may probably determine the clinical outcome of the acute attack.

... slightly decreased the first 5 days in all attacks. Increased fibrinolysis was suggested by decreased **plasminogen** values in severe attacks. Fibrinogen degradation products were seen in 40% of the patients in **blood** and in 100% of the patients in the peritoneal fluid. The

36/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06300443 86241986

The proteinase inhibitor complexes (antithrombin III-thrombin, alpha 2antiplasmin-plasmin and alpha 1antitrypsin-elastase) in septicemia, fulminant hepatic failure and cardiac shock: value for diagnosis and therapy control in DIC/F syndrome.

Egbring R; Seitz R; Blanke H; Leititis J; Kesper HJ; Burghard R; Fuchs G; Lerch L

Behring Institute Mitteilungen (GERMANY) Feb 1986, (79) p87-103, ISSN 0301-0457 Journal Code: 9KI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Thrombin (Thr), plasmin (Pl) and elastase (ELP) are serine proteinases which are quickly inactivated by their specific inhibitors (AT III, alpha 2AP, alpha 1AT), if intravascular activation of coagulation and fibrinolytic system or if release from PMN granulocytes by different stimuli (F.I., endotoxin, activated factor XII, a.o.) occurs. The immunological determination of the developing proteinase inhibitor complexes (PIC) AT III-Thr, alpha 2AP-Pl and alpha 1AT-ELP gives information as to whether intravascular coagulation, hyperfibrinolysis or unspecific proteolysis induced by elastase have taken place. Despite the high antiprotease activity in the plasma the a.m. serine proteinases may exert their proteolytic activity towards their specific substrates in vivo. In infectious diseases, fulminant hepatic failure and cardiac shock a complex consumption of coagulation factors and inhibitors may cause severe coagulation defects, microcirculatory disturbances and bleeding tendency. The PICs behaviour was determined in more than 80 patients with infectious diseases, in 5 patients with fulminant hepatic failure (FHF) and 7 patients with cardiac shock. Only in infectious diseases, mainly in septic complications, and septic complications during FHF and cardiac shock, are alpha 1AT-ELP levels found to be highly elevated. After cardiac shock, in FHF and in infectious diseases coagulation and fibrinolysis may additionally be activated. In this case AT III-Thr and alpha 2AP-Pl complexes could be detected in the patients plasma. This indicates that intravascular coagulation and hyperfibrinolysis has additionally taken place. To prevent bleeding complications a replacement therapy with plasma derivatives (AT III, **plasminogen** concentrate, PPSB and FFP) has been successfully performed in several patients with septic complications and in the 5 patients with FHF and the 7 patients with cardiac shock. No bleeding complication occurred, and the haemostatic balance could be maintained in the treated patients. AT III replacement therapy is necessary to stop DIC, PPSB improves severe coagulation defects, only FFP may additionally provide alpha 1AT, alpha 2AP and factor V. In acute renal failure sometimes **plasminogen** replacement is necessary to maintain a normal activity of the fibrinolytic system. The complex consumption of coagulation proteins in infectious diseases, FHF and cardiac shock cannot successfully be treated with an anticoagulant such as heparin alone.

... additionally taken place. To prevent bleeding complications a replacement therapy with plasma derivatives (AT III, **plasminogen** concentrate, PPSB and FFP) has been successfully performed in several patients with septic complications and...

... may additionally provide alpha 1AT, alpha 2AP and factor V. In acute renal failure sometimes **plasminogen** replacement is necessary to maintain a normal activity of the fibrinolytic system. The complex

consumption...
Descriptors: Disseminated Intravascular Coagulation--Complications--CO; *Liver Diseases--Diagnosis--DI; *Protease Inhibitors--**Blood**--BL; *Septicemia--Diagnosis--DI; *Shock, Cardiogenic--Diagnosis--DI...; Analysis --AN; Aged; Antiplasmin--Analysis--AN; Antithrombin III--Analysis--AN; Child, Preschool; Disseminated Intravascular Coagulation--**Blood**--BL; Disseminated Intravascular Coagulation--Therapy--TH; Liver Diseases--**Blood**--BL; Pancreatic Elastase--**Blood**--BL; Plasmin--Analysis --AN; Septicemia--**Blood**--BL; Shock, Cardiogenic--**Blood**--BL; Syndrome; Thrombin--Analysis--AN
Chemical Name: Pancreatic Elastase; (Thrombin; (Plasmin; (alpha 1-Antitrypsin; (plasmin-alpha(2)-antiplasmin **complex**; (Antiplasmin; (**Protease Inhibitors**; (Antithrombin III

36/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05819206 87177775
Disseminated intravascular coagulation and antiprotease activity in acute human pancreatitis.
Lasson A; Ohlsson K
Scandinavian journal of gastroenterology. Supplement (NORWAY) 1986, 126 p35-9, ISSN 0085-5928 Journal Code: UCT
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The coagulation and fibrinolytic systems were analysed parallel to the clinical evaluation in 27 attacks of acute human pancreatitis of different severity. Consumptive coagulopathy was evident from decreased platelet counts, decreased prothrombin values and consumption of fibrinogen during the first days in severe attacks. Fibrinolysis was suggested by decreased **plasminogen** values and the presence of fibrinogen degradation products. All main **protease inhibitors** of the two systems showed **protease**-antiprotease **complexation** and lower functional than quantitative values. Functional levels of the **protease inhibitors** were almost zero in the peritoneal fluid in severe attacks. It is concluded that severe acute pancreatitis results in consumptive coagulopathy and fibrinolysis together with a local antiprotease deficiency. All the changes are closely correlated to the

36/3,K,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08697209 95393613

Ligand western blotting for specific detection of active forms of proteases.

Kido T; Yook HY; Ueda K

Division of Medical Technology, College of Medical Technology, Kyoto University, Japan.

Clinica chimica acta; international journal of clinical chemistry (NETHERLANDS) Jun 15 1995, 237 (1-2) p31-41, ISSN 0009-8981
Journal Code: DCC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We developed a non-radioactive method of ligand western blotting for specific detection of active forms of serine proteases. The method consists of three steps: (i) separation of proteins by electrophoresis in sodium dodecyl sulfate-polyacrylamide gel, followed by blotting of proteins to nitrocellulose membrane; (ii) binding of a specific ligand, such as soybean trypsin inhibitor labeled with biotin, to **protease** on the membrane; and (iii) detection of the **protease-inhibitor complex** by color reaction (or chemiluminescence) developed by streptavidin-conjugated peroxidase (or alkaline phosphatase). By using this method, plasmin and trypsin (serine proteases) were detected, but papain (thiol protease) or pepsin (acidic protease) was not. Plasmin was detectable up to less than 4 ng. Inactive precursors of serine protease, i.e. **plasminogen** and trypsinogen, did not exhibit visible bands until they were activated by treatment with streptokinase or trypsin, respectively. We applied this method to clinical samples, and succeeded in detecting **plasminogen**, after conversion to plasmin with streptokinase treatment, in as little as 5 microliters of **serum** or trypsin, as it was in 10 microliters of

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? ds

Set     Items   Description
S1     149630   PROTEASE??
S2    1197232   INHIBITOR??
S3    3784523   EXTRACELLULAR OR SERUM OR BLOOD
S4      67044   S1 AND S2
S5    1579896   COMPLEX?
S6      10836   S4 AND S5
S7       3337   S6 AND S3
S8     353292   TARGET
S9        160   S7 AND S8
S10     46128   PROTEASE(5N)INHIBITOR??
S11     42282   PROTEASE(W)INHIBITOR??
S12     42282   S1 AND S11
S13      5829   S12 AND S5
S14    954464   SERUM
S15       665   S13 AND S14
S16      1469   PROTEASE(5N) COMPLEX?(5N)PROTEASE(W)INHIBITOR??
S17       212   S16 AND S14
S18     23319   CATHEPSIN??
S19        56   S16 AND S18
S20        42   RD (unique items)
S21         7   S20 AND S3
S22     61161   PLASMINOGEN(W)ACTIVATOR
S23       139   S16 AND S22
S24        55   S23 AND S3
S25        41   RD (unique items)
S26     36847   COLLAGENASE
S27        31   S16 AND S26
S28        28   RD (unique items)
S29        13   S28 AND S3
S30      6484   STROMELYSIN??
S31         0   S16 AND S30
S32     74806   PLASMINOGEN
S33       161   S16 AND S32
S34        67   S33 AND S3
S35        49   RD (unique items)
? s s35 not s22

            49  S35
         61161  S22
    S36      9  S35 NOT S22
? t s36/3,k,ab/1-9


 36/3,K,AB/1     (Item 1 from file: 155)
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? s prodrug

      S1   12096  PRODRUG
? s prepro?

      S2   26884  PREPRO?
? s s1 and s2

           12096  S1
           26884  S2
      S3       3  S1 AND S2
? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S4       1  RD (unique items)
? t s4/3,k,ab/1


 4/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09220781   96400327   PMID: 8806703
      Expression and characterization of human pancreatic
**preprocarboxypeptidase** A1 and **preprocarboxypeptidase** A2.
  Laethem RM; Blumenkopf TA; Cory M; Elwell L; Moxham CP; Ray PH; Walton LM
; Smith GK
  Division of Cell Biology, Wellcome Research Laboratories, Research
Triangle Park, North Carolina 27709, USA.
  Archives of biochemistry and biophysics (UNITED STATES)  Aug 1 1996,
332 (1) p8-18, ISSN 0003-9861  Journal Code: 6SK
  Languages: ENGLISH
  Document type: Journal Article
  Record type: Completed
  We are investigating the potential utility of human carboxypeptidases A
in antibody-directed enzyme **prodrug** therapy (ADEPT). Hybridization
screening of a human pancreatic cDNA library with cDNA probes that encoded
either rat carboxypeptidase A1 (rCPA1) or carboxypeptidase A2 (rCPA2) was
used to clone the human **prepro**-CPA homologs. After expression of the
respective pro-hCPA cDNA in Saccharomyces cerevisiae, the enzymes were
purified to homogeneity by a combination of hydrophobic and ion-exchange
chromatography. Purified hCPA1 and hCPA2 migrate as a single protein band
with M(r) 34,000 when subjected to gel electrophoresis in the presence of
sodium dodecyl sulfate under reducing conditions. Kinetic studies of the
purified enzymes with hippuryl-L-phenylalanine resulted in kcat/Km values
of 57,000 and 19,000 M-1 s-1 for hCPA1 and hCPA2, respectively. Using the
ester substrate, hippuryl-D, L-phenyllactate, we found unique esterase/
peptidase specific activity ratios among hCPA1, hCPA2, rCPA1, and bovine
CPA (bCPA) ranging from 13 to 325. Two potential ADEPT substrates,
methotrexate-alpha-phenylalanine (MTX-Phe) and methotrexate-alpha-(1-naphth
yl)alanine (MTX-naphthylAla) were also analyzed. The kcat/Km values for
MTX-Phe were 440,000 and 90,000 M-1 s-1 for hCPA1 and hCPA2, respectively,
and for MTX-naphthylAla these values were 1400 and 1,400,000 M-1 s-1 for
hCPA1 and hCPA2, respectively. The kinetic data show that hCPA2 has a
larger substrate binding site than the hCPA1 enzyme. Differences between
hCPA1 and hCPA2 were also observed in thermal stability experiments at 60
degrees C where the half-life for thermal denaturation of hCPA2 is
eightfold longer than that for hCPA1. These experiments indicate that hCPA1
and hCPA2 are potential candidates for use in a human-based ADEPT approach.

      Expression and characterization of human pancreatic
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preprocarboxypeptidase A1 and **preprocarboxypeptidase** A2.

We are investigating the potential utility of human carboxypeptidases A in antibody-directed enzyme **prodrug** therapy (ADEPT). Hybridization screening of a human pancreatic cDNA library with cDNA probes that encoded either rat carboxypeptidase A1 (rCPA1) or carboxypeptidase A2 (rCPA2) was used to clone the human **prepro**-CPA homologs. After expression of the respective pro-hCPA cDNA in Saccharomyces cerevisiae, the enzymes...

Enzyme No.: EC 3.4.- (Carboxypeptidases); EC 3.4.17.- (**preprocarboxypeptidase** A)

Chemical Name: DNA Primers; Enzyme Precursors; Prodrugs; methotrexate-alpha-phenylalanine; Phenylalanine; Methotrexate; N-(hippuryl) phenylalanine; Carboxypeptidases; **preprocarboxypeptidase** A

?

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Set     Items   Description
S1      12085   PRODRUG
S2       2803   PROTEASE(5N)SUBSTRATE
S3          1   S1 AND S2
? s protease

      S4  134898  PROTEASE
? s s1 and s2

           12085  S1
            2803  S2
      S5       1  S1 AND S2
? s s1 and s4

           12085  S1
          134898  S4
      S6     196  S1 AND S4
? s cleav?

      S7  220182  CLEAV?
? s s6 and s7

             196  S6
          220182  S7
      S8      21  S6 AND S7
? rd

>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S9      14  RD (unique items)
? t s9/3,k,ab/1-14


 9/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11365070   21301254   PMID: 11408178
  Design, synthesis, and biological evaluation of anti-HIV double-drugs.
conjugates of HIV protease inhibitors with a reverse transcriptase
inhibitor through spontaneously cleavable linkers.
  Matsumoto H; Kimura T; Hamawaki T; Kumagai A; Goto T; Sano K; Hayashi Y;
Kiso Y
  Department of Medicinal Chemistry, Center for Frontier Research in
Medicinal Science, Kyoto Pharmaceutical University, Yamashina-ku, 607-8412,
Kyoto, Japan
  Bioorganic & medicinal chemistry (England)  Jun 2001,  9  (6)  p1589-600
,  ISSN 0968-0896  Journal Code: B38
  Languages: ENGLISH
  Document type: Journal Article
  Record type: In Process
  Based on the prodrug concept as well as the combination of two
different classes of anti-HIV agents, we designed and synthesized a series
of anti-HIV double-drugs consisting of HIV protease inhibitors
conjugated with a nucleoside reverse transcriptase inhibitor in an effort
to enhance the antiviral activity. For the conjugation, a series of linkers
that conjoins the two different classes of inhibitors has been
investigated. Double-drugs using a succinyl amino acid linker were shown to
release the parent drugs via spontaneous imide formation at a faster rate
compared to compounds using a glutaryl amino acid linker, as expected from
the energetically favorable cyclization to the five-membered ring. Among
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the double-drugs, KNI-1039 (3b) with a glutarylglycine linker exhibited extremely potent anti-HIV activity compared with that of the individual components. Double-drug 3b was relatively stable in culture medium, whereas it regenerated active species in cell homogenate. These results suggested that the synergistic enhancement of anti-HIV activities of 3b may be due to their ability to penetrate into the target cell and subsequent regeneration of two different classes of anti-HIV agents in the cytoplasm.

Design, synthesis, and biological evaluation of anti-HIV double-drugs. conjugates of HIV **protease** inhibitors with a reverse transcriptase inhibitor through spontaneously **cleavable** linkers.

Based on the **prodrug** concept as well as the combination of two different classes of anti-HIV agents, we designed and synthesized a series of anti-HIV double-drugs consisting of HIV **protease** inhibitors conjugated with a nucleoside reverse transcriptase inhibitor in an effort to enhance the antiviral...

9/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10831037 20322756 PMID: 10866387

Double-Drugs'--a new class of **prodrug** form of an HIV **protease** inhibitor conjugated with a reverse transcriptase inhibitor by a spontaneously **cleavable** linker.

Matsumoto H; Hamawaki T; Ota H; Kimura T; Goto T; Sano K; Hayashi Y; Kiso Y

Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Japan.

Bioorganic & medicinal chemistry letters (ENGLAND) Jun 5 2000, 10 (11) p1227-31, ISSN 0960-894X Journal Code: C8B

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

We designed and synthesized a new series of **prodrug**-type anti-HIV agents consisting of a peptidomimetic HIV **protease** inhibitor conjugated with a nucleoside reverse transcriptase inhibitor in an effort to enhance the antiviral activity. For the conjugation, a series of linkers that conjoin the two different classes of inhibitors have been investigated. Conjugates using a succinyl amino acid linker were shown to release the parent components via the spontaneous imide formation at a faster rate compared to conjugates using a glutaryl amino acid linker, as expected from the energetically favorable cyclization to the five-membered ring. Herein, we report a new 'double-drug' 4b (KNI-1039) with a glutarylglycine linker, which exhibited extremely potent anti-HIV activity compared with that of the individual components.

Double-Drugs'--a new class of **prodrug** form of an HIV **protease** inhibitor conjugated with a reverse transcriptase inhibitor by a spontaneously **cleavable** linker.

We designed and synthesized a new series of **prodrug**-type anti-HIV agents consisting of a peptidomimetic HIV **protease** inhibitor conjugated with a nucleoside reverse transcriptase inhibitor in an effort to enhance the antiviral...

Descriptors: Anti-HIV Agents--chemistry--CH; *HIV **Protease** Inhibitors--chemistry--CH; *Prodrugs--chemistry--CH; *Reverse Transcriptase Inhibitors--chemistry--CH; *Thiazoles--chemistry--CH; *Zidovudine...

Chemical Name: Anti-HIV Agents; HIV **Protease** Inhibitors; KNI 1039; Prodrugs; Reverse Transcriptase Inhibitors; Thiazoles; Zidovudine

9/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09500865 96009647 PMID: 7547890
Maduropeptin: an antitumor chromoprotein with selective **protease** activity and DNA **cleaving** properties.
Zein N; Solomon W; Colson KL; Schroeder DR
Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000, USA.
Biochemistry (UNITED STATES) Sep 12 1995, 34 (36) p11591-7, ISSN 0006-2960 Journal Code: A0G
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Maduropeptin (MDP) is a recently isolated antitumor antibiotic, consisting of an enediyne-containing chromophore embedded in a highly acidic protein. This holoantibiotic damages duplex DNA in vitro, producing a mixture of single- and double-strand breaks at selected sites. The chromophore, isolated as the methanol adduct from the protein-containing holoantibiotic, exhibits the same selectivity and **cleavage** chemistry as the chromoprotein complex. Preliminary evidence suggests that the primary DNA breaks involve 4'-H abstraction from the deoxyribose sugars at the **cleavage** sites. Unlike most other enediyne antitumor antibiotics, DNA strand scission is not bioreductively induced by MDP or the methanol adduct of the chromophore. This was also observed for the C1027 chromophore. DNA **cleavage** is inhibited in the presence of certain cations (Ca2+, Mg2+) as was observed with the kedarcidin chromophore. 1H NMR spectroscopy studies on the methanol adduct of the maduropeptin chromophore in the presence of calcium chloride provide clues regarding its activation and give insight as to the regions of the chromophore important for DNA binding. Our results suggest that the solvent artifact of the chromophore may in essence be a **prodrug** and it regenerates the parent chromophore as in the holoantibiotic prior to **cleaving** DNA. As with kedarcidin and neocarzinostatin, maduropeptin exhibits a high affinity for histones, in vitro, **cleaving** them to low molecular mass peptides. Histone H1, the most opposite in net charge, is **cleaved** most readily. This latter activity may serve to disrupt the chromatin superstructure in vivo, prior to exposing the DNA to the chromophore.

Maduropeptin: an antitumor chromoprotein with selective **protease** activity and DNA **cleaving** properties.
... isolated as the methanol adduct from the protein-containing holoantibiotic, exhibits the same selectivity and **cleavage** chemistry as the chromoprotein complex. Preliminary evidence suggests that the primary DNA breaks involve 4'-H abstraction from the deoxyribose sugars at the **cleavage** sites. Unlike most other enediyne antitumor antibiotics, DNA strand scission is not bioreductively induced by...

... the methanol adduct of the chromophore. This was also observed for the C1027 chromophore. DNA **cleavage** is inhibited in the presence of certain cations (Ca2+, Mg2+) as was observed with the...

... Our results suggest that the solvent artifact of the chromophore may in essence be a **prodrug** and it regenerates the parent chromophore as in the holoantibiotic prior to **cleaving** DNA. As with kedarcidin and neocarzinostatin, maduropeptin exhibits a high affinity for histones, in vitro, **cleaving** them to low molecular mass peptides. Histone H1, the most opposite in net charge, is **cleaved** most readily. This latter activity may serve to disrupt the chromatin superstructure in vivo, prior ...

9/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08266814 95032183 PMID: 7945439
Phospholipid **prodrug** inhibitors of the HIV **protease** .

Antiviral activity and pharmacokinetics in rats.

Hostetler KY; Richman DD; Forssen EA; Selk L; Basava R; Gardner MF; Parker S; Basava C

Department of Medicine, University of California, San Diego, La Jolla 92093.

Biochemical pharmacology (ENGLAND) Oct 7 1994, 48 (7) p1399-404, ISSN 0006-2952 Journal Code: 9Z4

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The aspartyl **protease** of the human immunodeficiency virus (HIV) is an important target for chemotherapeutic intervention because of its key role in **cleaving** the HIV gag-pol polyprotein during viral assembly and budding. Short peptides and peptidomimetics, which bind to the active site of the HIV aspartyl **protease** and inhibit processing of the polyprotein, have been synthesized. These compounds are active against HIV in vitro, but many face substantial development problems because of their rapid elimination from the body in bile and urine. Refinement of these agents appears to be necessary if they are to become useful clinically. Recently, we developed a novel chemical strategy for increasing plasma levels of HIV **protease** inhibitory peptides, which involves the attachment of a biodegradable phospholipid group to the C-terminus of a pentapeptide, iBOC-[L-Phe]-[D-beta-Nal]-Pip-[alpha-(OH)-Leu]-Val (7194). We coupled phosphatidylethanolamine to the C-terminal valine of 7194 to make a phospholipid **prodrug** (7196). In vitro assays in HT4-6C cells infected with HIV-1 showed that the antiviral activity of the C-terminal phospholipid **prodrug** , 7196, was equal to that of the free peptide, 7194. Similar results were obtained in vitro when a related pentapeptide (7140) was derivatized at the N-terminal with dipalmitoylphosphatidylethano lamine-succinic acid (7172). Tritium-labeled 7194 and 7196 were prepared and injected intravenously into rats at 3 mumol/kg; then the plasma was assayed for native compound and metabolites by HPLC radioactivity flow detection. The peak plasma level of the tritium-labeled lipid **prodrug** (7196) was 36 microM versus 1.6 microM for the free **protease** inhibitor pentapeptide (7194). The area under the curve of the phospholipid **prodrug** (7196) was 48-fold greater and its mean residence time was increased 43-fold versus the free peptide (7194). Phospholipid prodrugs appear to offer an alternative approach to optimizing in vivo performance of HIV **protease** inhibitors and other small peptides.

Phospholipid **prodrug** inhibitors of the HIV **protease** . Antiviral activity and pharmacokinetics in rats.

The aspartyl **protease** of the human immunodeficiency virus (HIV) is an important target for chemotherapeutic intervention because of its key role in **cleaving** the HIV gag-pol polyprotein during viral assembly and budding. Short peptides and peptidomimetics, which bind to the active site of the HIV aspartyl **protease** and inhibit processing of the polyprotein, have been synthesized. These compounds are active against HIV ...

... useful clinically. Recently, we developed a novel chemical strategy for increasing plasma levels of HIV **protease** inhibitory peptides, which involves the attachment of a biodegradable phospholipid group to the C-terminus...

... 7194). We coupled phosphatidylethanolamine to the C-terminal valine of 7194 to make a phospholipid **prodrug** (7196). In vitro assays in HT4-6C cells infected with HIV-1 showed that the antiviral activity of the C-terminal phospholipid **prodrug**, 7196, was equal to that of the free peptide, 7194. Similar results were obtained in...

... metabolites by HPLC radioactivity flow detection. The peak plasma level of the tritium-labeled lipid **prodrug** (7196) was 36 microM versus 1.6 microM for the free **protease** inhibitor pentapeptide (7194). The area

under the curve of the phospholipid **prodrug** (7196) was 48-fold greater and its mean residence time was increased 43-fold versus...

... Phospholipid prodrugs appear to offer an alternative approach to optimizing in vivo performance of HIV **protease** inhibitors and other small peptides.

Descriptors: Antiviral Agents--pharmacology--PD; *HIV **Protease** Inhibitors--pharmacology--PD; *HIV-1; *Phospholipids--pharmacology--PD; *Prodrugs--pharmacology--PD; Amino Acid Sequence; Antiviral Agents --pharmacokinetics--PK; HIV **Protease** Inhibitors--pharmacokinetics--PK ; Half-Life; Hela Cells; Metabolic Clearance Rate; Molecular Sequence Data; Oligopeptides--pharmacokinetics...

Chemical Name: Antiviral Agents; HIV **Protease** Inhibitors; Oligopeptides; Phosphatidylethanolamines; Phospholipids; Prodrugs; pentapeptide 7194; phospholipid **prodrug** 7196

9/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05995229 87011337 PMID: 2945003

Inhibition of Met5-enkephalin-Arg6-Phe7 degradation by inhibitors of dipeptidyl carboxypeptidase.

Mellstrom B; Iadarola MJ; Yang HY; Costa E

Journal of pharmacology and experimental therapeutics (UNITED STATES) Oct 1986, 239 (1) p174-8, ISSN 0022-3565 Journal Code: JP3

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The heptapeptide Met5-enkephalin-Arg6-Phe7 (YGGFMRF) is **cleaved** at a high rate by tissue peptidases including dipeptidyl carboxypeptidase. The inhibitor, Hoe 498 diacid (2-[N-[(S)-1-carboxy-3-phenylpropyl]-L-alanyl]-(1 S,3S,5S)-2-azabicyclo- [3.3.0] octane-3-carboxylic acid), was found to be highly effective in blocking YGGFMRF degradation by a dipeptidyl carboxypeptidase present in a preparation of mouse striatal microsomes. The recovery of YGGFMRF released from rat striatal slices was increased in the presence of Hoe 498 diacid. Furthermore, the recovery of YGGFMRF injected into the caudate was increased in rats pretreated i.p. with Hoe 498 diacid. After i.v. or i.p. injections both Hoe 498 diacid and its **prodrug** Hoe 498 monoester (2-[N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl]-(1S,3S, 5S)-2- azabicyclo [3.3.0] octane-3-carboxylic acid) were detected in rat cerebrospinal fluid and the dipeptidyl carboxypeptidase activity in cerebrospinal fluid was inhibited. These observations indicate that endogenously released YGGFMRF is protected from degradation by Hoe 498 diacid and that systemically administered Hoe 498 diacid or monoester penetrate the blood-brain barrier and inhibit brain and cerebrospinal fluid dipeptidyl carboxypeptidase activity. This potent inhibitor may be useful to block YGGFMRF inactivation in studies of the pharmacology and physiology of YGGFMRF. It is also possible that some of the cerebral effects of these compounds may be referable to an enhancement of YGGFMRF action in the central nervous system.

The heptapeptide Met5-enkephalin-Arg6-Phe7 (YGGFMRF) is **cleaved** at a high rate by tissue peptidases including dipeptidyl carboxypeptidase. The inhibitor, Hoe 498 diacid...

...498 diacid. After i.v. or i.p. injections both Hoe 498 diacid and its **prodrug** Hoe 498 monoester (2-[N-[(S)-1-ethoxycarbonyl-3-phenylpropyl] -L-alanyl]-(1S,3S,5S...

Descriptors: Enkephalin, Methionine--analogs and derivatives--AA; * **Protease** Inhibitors

Chemical Name: Bicyclo Compounds; **Protease** Inhibitors; Enkephalin, Methionine; enkephalin-Met, Arg(6)-Phe(7)-; Ramipril; Endopeptidases; dipeptidyl carboxypeptidase

9/3,K,AB/6 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

12963326 BIOSIS NO.: 200100170475
Controlled drug release: New water-soluble prodrugs of an HIV **protease** inhibitor.
AUTHOR: Matsumoto Hikaru; Sohma Youhei; Kimura Tooru; Hayashi Yoshio; Kiso Yoshiaki(a)
AUTHOR ADDRESS: (a)Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto, 607-8412: kiso@mb.kyoto-phu.ac.jp**Japan
JOURNAL: Bioorganic & Medicinal Chemistry Letters 11 (4):p605-609 26 February, 2001
MEDIUM: print
ISSN: 0960-894X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: We designed and synthesized a series of highly water-soluble prodrugs of an HIV **protease** inhibitor, KNI-727 (1), containing tandem-linked two auxiliary units, a solubilizing moiety and a self-**cleavable** spacer. Prodrugs with an ionized amino group at the solubilizing moiety exhibited a remarkable increase of water-solubility (>104 fold) compared to the parent drug 1. These prodrugs released 1 not enzymatically, but chemically via an intramolecular cyclization-elimination reaction through an imide formation in physiological conditions. Diversified rates of parent drug release were observed when the chemical structure of both the solubilizing and the spacer moieties were modified. This new approach for water-soluble prodrugs will enable to control chemically the release of parent drug as well as to maintain high water-solubility.

2001

Controlled drug release: New water-soluble prodrugs of an HIV **protease** inhibitor.

ABSTRACT: We designed and synthesized a series of highly water-soluble prodrugs of an HIV **protease** inhibitor, KNI-727 (1), containing tandem-linked two auxiliary units, a solubilizing moiety and a self-**cleavable** spacer. Prodrugs with an ionized amino group at the solubilizing moiety exhibited a remarkable increase...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...**protease** inhibitor-drug, release...

...KNI-727 **prodrug**--

9/3,K,AB/7 (Item 2 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

12653641 BIOSIS NO.: 200000407143
Bioadhesive peptides as potential anticancer drug carriers: Activation via isopeptide deblocking by proteases.
AUTHOR: Yamazaki Yoshimitsu(a); Tsuruga Mie; Gentsch Bill; Oka Syuichi; Kleinman Hynda K; Mokotoff Michael
AUTHOR ADDRESS: (a)National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, 1-1 Higashi, Tsukuba,

Ibaraki, 305-8566**Japan
JOURNAL: Anticancer Research 20 (3A):p1381-1384 May-June, 2000
MEDIUM: print
ISSN: 0250-7005
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Background: Bioadhesive peptides are potentially useful as anticancer drug carriers, if the bioadhesivity becomes active only at the tumor site. We propose that this function can be achieved by the **prodrug** strategy using proteases in tumors. Materials and Methods: A laminin fragment peptide, Ac-AASIKVAVSADR-NH2 (5), and its derivatives, Ac-AASIK(L) VAVSADR-NH2 (6), Ac-AASIDpm(NH2)VAVSADR-NH2 (7) and Ac-AASIDpm(L)(NH2)VAVSADR-NH2 (8), were synthesized and tested for their bioadhesive activity with 6 cancer cell lines. Results: The strength of the binding was in the order of 5 mchgt 7 gtoreq 8 gtoreq 6. The attachment of mouse whole blood cells to peptide 6-coated surface was also weaker than to 5-coated surface. The Leu isopeptide linkage in 6 was enzymatically **cleaved** by the cells. Conclusion: The present results suggest that isopeptide 6, working as a **prodrug** form for the bioadhesive peptide 5, could serve as an anticancer drug carrier for tumor targeting.

2000

...ABSTRACT: only at the tumor site. We propose that this function can be achieved by the **prodrug** strategy using proteases in tumors. Materials and Methods: A laminin fragment peptide, Ac-AASIKVAVSADR-NH2...

...also weaker than to 5-coated surface. The Leu isopeptide linkage in 6 was enzymatically **cleaved** by the cells. Conclusion: The present results suggest that isopeptide 6, working as a **prodrug** form for the bioadhesive peptide 5, could serve as an anticancer drug carrier for tumor...
...REGISTRY NUMBERS: **PROTEASE**
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...**protease**

9/3,K,AB/8 (Item 3 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

09577074 BIOSIS NO.: 199598031992
Phospholipid **prodrug** inhibitors of the HIV **protease**.
AUTHOR: Hostetler Karl Y; Richman Douglas D; Forssen Eric A; Selk Linda; Basava Rathna; Gardner Michael F; Parker Suezanne; Basava Channa
AUTHOR ADDRESS: Dep. Med., Univ. California, San Diego, Clinical Sci. Build., Room 305, 9500 Gilman Drive, La Jolla,**USA
JOURNAL: Biochemical Pharmacology 48 (7):p1399-1404 1994
ISSN: 0006-2952
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The aspartyl **protease** of the human immunodeficiency virus (HIV) is an important target for chemotherapeutic intervention because of its key role in **cleaving** the HIV gag-pol polyprotein during viral assembly and budding. Short peptides and peptiomimetics, which bind to the active site of the HIV aspartyl **protease** and inhibit processing of the polyprotein. have been synthesized. These compounds are active against HIV in vitro, but may face substantial development problems

because of their rapid elimination from the body in bile and urine. Refinement of these agents appears to be necessary if they are to become useful clinically. Recently, we developed a novel chemical strategy for increasing plasma levels of HIV **protease** inhibitory peptides, which involves the attachment of a biodegradable phospholipid group to the C-terminus or a pentapeptide, iBOC-(L-Phe)-(D-beta-Nal)-Pip(alpha-(OH)-Leu)-Val (7194). We coupled phosphatidylethanolamine to the C-terminal valine of 7194 to make a phospholipid **prodrug** (7196). In vitro assays in HT4-6C cells infected with HIV-1 showed that the antiviral activity of the C-terminal phospholipid **prodrug**, 7196, was equal to that of the free peptide, 7194. Similar results were obtained in vitro when a related pentapeptide (7140) was derivatized at the N-terminal with dipalmitoylphosphatidylethanolamine-succinic acid (7172). Tritium-labeled 7194 and 7196 were prepared and injected intravenously into rats at 3 mu-mol/kg; then the plasma was assayed for native compound and metabolites by HPLC radioactivity flow detection. The peak plasma level of the tritium-labeled lipid **prodrug** (7196) was 36 mu-M versus 1.6 mu-M for the free **protease** inhibitor pentapeptide (7194). The area under the curve of the phospholipid **prodrug** (796) was 48-fold greater and its mean residence time was increased 43-fold versus the free peptide (7194). Phospholipid prodrugs appear to offer an alternative approach to optimizing in vivo performance of HIV **protease** inhibitors and other small peptides.

1994

Phospholipid **prodrug** inhibitors of the HIV **protease**.

ABSTRACT: The aspartyl **protease** of the human immunodeficiency virus (HIV) is an important target for chemotherapeutic intervention because of its key role in **cleaving** the HIV gag-pol polyprotein during viral assembly and budding. Short peptides and peptiomimetics, which bind to the active site of the HIV aspartyl **protease** and inhibit processing of the polyprotein. have been synthesized. These compounds are active against HIV...

...useful clinically. Recently, we developed a novel chemical strategy for increasing plasma levels of HIV **protease** inhibitory peptides, which involves the attachment of a biodegradable phospholipid group to the C-terminus...

...7194). We coupled phosphatidylethanolamine to the C-terminal valine of 7194 to make a phospholipid **prodrug** (7196). In vitro assays in HT4-6C cells infected with HIV-1 showed that the antiviral activity of the C-terminal phospholipid **prodrug**, 7196, was equal to that of the free peptide, 7194. Similar results were obtained in...

...metabolites by HPLC radioactivity flow detection. The peak plasma level of the tritium-labeled lipid **prodrug** (7196) was 36 mu-M versus 1.6 mu-M for the free **protease** inhibitor pentapeptide (7194). The area under the curve of the phospholipid **prodrug** (796) was 48-fold greater and its mean residence time was increased 43-fold versus...

...Phospholipid prodrugs appear to offer an alternative approach to optimizing in vivo performance of HIV **protease** inhibitors and other small peptides.
...REGISTRY NUMBERS: **PROTEASE**
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: **PROTEASE**

9/3,K,AB/9 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENT

Dialog Acc No: 3294051 IFI Acc No: 0008103
Document Type: C
COMPOSITIONS CONTAINING NUCLEIC ACIDS AND LIGANDS FOR THERAPEUTIC TREATMENT ; GENE DELIVERY COMPOSITION COMPRISING POLYPEPTIDE THAT BINDS TO FIBROBLAST GROWTH FACTOR RECEPTOR-NUCLEIC ACID BINDING DOMAIN-NUCLEIC ACID MOLECULE
Inventors: Baird J Andrew (US); Chandler Lois Ann (US); Sosnowski Barbara A (US)
Assignee: Selective Genetics Inc Assignee Code: 52789
Patent (No,Date), Applic (No,Date)
US 6037329 20000314 US 96718904 19960924
Calculated Expiration: 20140315
Cont.-in-part Pat(No),Applic(No,Date): ABANDONED US 94213446 19940315; ABANDONED US 94213447 19940315; ABANDONED US 94297961 19940829; ABANDONED US 94305771 19940913; ABANDONED US 95441979 19950516
Priority Applic(No,Date): US 96718904 19960924; US 94213446 19940315; US 94213447 19940315; US 94297961 19940829; US 94305771 19940913; US 95441979 19950516

Abstract:
Preparations of conjugates of a receptor-binding internalized ligand and a cytocide-encoding agent and compositions containing such preparations are provided. The conjugates contain a polypeptide that is reactive with an FGF receptor, such as bFGF, or another heparin-binding growth factor, cytokine, or growth factor coupled to a nucleic acid binding domain. One or more linkers may be used in the conjugation. The linker is selected to increase the specificity, toxicity, solubility, serum stability, or intracellular availability, and promote nucleic acid condensation of the targeted moiety. The conjugates are complexed with a cytocide-encoding agent, such as DNA encoding saporin. Conjugates of a receptor-binding internalized ligand to a nucleic acid molecule are also provided.

Non-exemplary Claims:
...8. The composition of claim 1, wherein the nucleic acid molecule is a **prodrug**-encoding agent...

...15. The composition of claim 8 wherein the **prodrug**-encoding agent further comprises a tissue-specific promoter operably linked thereto... comprising between one to six linkers that are selected from the group consisting of a **cleavable** linker, a linker that provides a sorting signal, a linker that reduces steric hindrance and...
...or the formula: polypeptide that binds to an FGF receptor-L-nucleic acid binding domain-**prodrug** encoding agent wherein: L is one to six linkers; and wherein the conjugate retains the...

...bind to an FGF receptor and internalize the nucleic acid molecule, cytocide-encoding agent or **prodrug** encoding agent, and wherein the cytocide-encoding agent is bound to the nucleic acid binding...33. The composition of claim 19, wherein the **cleavable** linker is **cleavable** by a **protease**.
...

...34. The composition of claim 33, wherein the **protease** is selected from the group consisting of cathepsin B, cathepsin D and trypsin...

...35. The composition of claim 8, wherein the **prodrug**-encoding agent encodes HSV-thymidine kinase or cytosine deaminase.

9/3,K,AB/10 (Item 2 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENT

Dialog Acc No: 2991904 IFI Acc No: 9818947
Document Type: C
SUBSTITUTED CYCLIC UREAS AND DERIVATIVES THEREOF USEFUL AS RETROVIRAL **PROTEASE** INHIBITORS
Inventors: Delucca George Vincent (US)
Assignee: Du Pont Merck Pharmaceutical Co Assignee Code: 25859
Patent (No,Date), Applic (No,Date)
  US 5763469 19980609 US 96701112 19960821
  Calculated Expiration: 20160821
Priority Applic(No,Date): US 96701112 19960821
Provisional Applic(No,Date): US 60-2653 19950823

Abstract:
The present invention relates to substituted cyclic ureas and analogs thereof of formula (I):

D R A W I N G

wherein, T is selected from: -N(R22)C(=Z)N(R23)-; N(R22)C(=Z)C(=Z)N(R23)-; -N(R22)S(=Z')N(R23)-; N(R22)S(=Z')2N(R23)-; or -N(R22)P(=O)(R24a)N(R23)-; Z is O, S, NR24; and, Z' is O or NR24; or pharmaceutically acceptable salt forms or prodrugs thereof, which are useful as retroviral **protease** inhibitors, and to pharmaceutical compositions comprising such compounds and methods of using the same for treating viral infection.

SUBSTITUTED CYCLIC UREAS AND DERIVATIVES THEREOF USEFUL AS RETROVIRAL **PROTEASE** INHIBITORS

Abstract:
...or NR24; or pharmaceutically acceptable salt forms or prodrugs thereof, which are useful as retroviral **protease** inhibitors, and to pharmaceutical compositions comprising such compounds and methods of using the same for...
Exemplary Claim:
...which carbon 2 of the ring is replaced with J)

or pharmaceutically acceptable salt or **prodrug** form thereof, wherein; J is C=O or C=S; R4 and R7 are independently...

...substituted with 0-3 R12; or any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl, amino or sulfhydryl; R22 and R23 are independently selected from...
Non-exemplary Claims:
...or pharmaceutically acceptable salt or **prodrug** form thereof, wherein; R4 and R7 are independently selected from the following: -NOR13R13; C1-6...

...substituted with 0-3 R12; or any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl, amino or sulfhydryl; m is 0, 1 or 2; R22...

...3. A compound according to claim 2 or pharmaceutically acceptable salt or **prodrug** form thereof, wherein; R4 and R7 are independently C1-4 alkyl substituted with 0-2...or halogen R20 is hydrogen or any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl; R22 and R23 are independently selected from the following: C1...

...or pharmaceutically acceptable salt or **prodrug** forms thereof, wherein; R5 and R6 are independently selected from the following:

hydrogen, hydroxy, F...

...5. A compound according to claim 4 or pharmaceutically acceptable salt or **prodrug** forms thereof, wherein; R5 and R6 are independently selected from H, OH, F, Br, or...6. A compound according to claim 5 or pharmaceutically acceptable salt or **prodrug** forms thereof, wherein: R5 and R6 are both F; R5 and R6 are both CH2OH...

...therapeutically effective amount of a compound of claim 1 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 2 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 3 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 4 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 5 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 6 or a pharmaceutically acceptable salt or **prodrug** form thereof...
...therapeutically effective amount of a compound of claim 1 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 2 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 3 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 4 or a pharmaceutically acceptable salt or **prodrug** form thereof...
therapeutically effective amount of a compound of claim 5 or a pharmaceutically acceptable salt or **prodrug** form thereof...

...therapeutically effective amount of a compound of claim 6 or a pharmaceutically acceptable salt or **prodrug** form thereof.

9/3,K,AB/11 (Item 3 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENT

Dialog Acc No: 2838480 IFI Acc No: 9712065
Document Type: C
YEAST STRAINS USED TO IDENTIFY INHIBITORS OF DIBASIC AMINO ACID PROCESSING ENDOPROTEASES; PRODUCTION OF PROTEINS WITH DIBASIC AMINO ACIDS AND **CLEAVAGE**
Inventors: Franzusoff Alex (US)
Assignee: Colorado, University of Assignee Code: 18813
Patent (No,Date), Applic (No,Date)
US 5627043 19970506 US 95437820 19950509
Calculated Expiration: 20140506
Division Pat(No),Applic(No,Date): US 5413914 US 9388322 19930707
Priority Applic(No,Date): US 95437820 19950509; US 9388322 19930707

Abstract:
The present invention relates to novel yeast strains which produce a heterologous precursor protein having a dibasic amino acid processing site

which can be processed into at least one **cleavage** protein by a dibasic amino acid processing **endoprotease**. Such novel yeast strains are useful for identifying compounds capable of inhibiting infectious agents, such as viruses, that depend upon dibasic amino acid processing **endoprotease cleavage** for effective propagation and/or infectivity.

...PRODUCTION OF PROTEINS WITH DIBASIC AMINO ACIDS AND **CLEAVAGE**

Abstract:
...having a dibasic amino acid processing site which can be processed into at least one **cleavage** protein by a dibasic amino acid processing **endoprotease**. Such novel yeast strains are useful for identifying compounds capable of inhibiting infectious agents, such as viruses, that depend upon dibasic amino acid processing **endoprotease cleavage** for effective propagation and/or infectivity.
Exemplary Claim:
...I N G

1. A yeast strain which produces a heterologous dibasic amino acid processing **endoprotease** and a heterologous precursor protein of an infectious agent, said precursor protein having a dibasic...

...acid processing site, wherein said yeast strain processes said precursor protein into at least one **cleavage** protein.
Non-exemplary Claims:
2. The yeast strain of claim 1, wherein said yeast strain comprises a Kex2 **endoprotease**-deficient yeast strain which produces a heterologous dibasic amino acid processing **endoprotease** which **cleaves** said precursor protein...

...of claim 2, wherein said yeast strain is deficient in at least one soluble vacuolar **protease** selected from the group consisting of proteinase A, proteinase B, and proteinase C...

...8. A Kex2 **endoprotease**-deficient yeast strain which produces a heterologous precursor protein having a dibasic amino acid processing site, wherein said yeast strain does not process said precursor protein into at least one **cleavage** protein...

...of claim 8, wherein said yeast strain is deficient in at least one soluble vacuolar **protease** selected from the group consisting of proteinase A, proteinase B, and proteinase C...

...11. A yeast strain which produces a heterologous dibasic amino acid processing **endoprotease**, wherein said **endoprotease** processes a precursor protein having a dibasic amino acid **cleavage** site into at least one **cleavage** protein...

...13. The yeast strain of claim 11, wherein said **endoprotease** processes a **prodrug** which has been endocytosed by said yeast, said **prodrug** having a dibasic amino acid processing site...

...14. The yeast strain of claim 11, wherein said yeast strain is a Kex2 **endoprotease**-deficient yeast strain...

...of claim 11, wherein said yeast strain is deficient in at least one soluble vacuolar **protease** selected from the group consisting of proteinase A, proteinase B, and proteinase C...

9/3,K,AB/12 (Item 4 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENT

Dialog Acc No: 2827557 IFI Acc No: 9708641
Document Type: C
METHOD OF TREATING HUMAN IMMUNODEFICIENCY VIRUS INFECTION USING A CYCLIC **PROTEASE** INHIBITOR IN COMBINATION WITH A REVERSE TRANSCRIPTASE INHIBITOR
Inventors: Otto Michael J (US)
Assignee: Du Pont Merck Pharmaceutical Co Assignee Code: 25859
Document Type: REASSIGNED
Patent (No,Date), Applic (No,Date)
US 5616578 19970401 US 93110603 19930826
Calculated Expiration: 20140401
Priority Applic(No,Date): US 93110603 19930826

Abstract:
This invention relates to a method of treating human immunodeficiency virus (HIV) infection in a mammal comprising administering to the mammal a therapeutically effective amount of a combination of: (i) at least one cyclic HIV **protease** inhibitor and (ii) at least one HIV reverse transcriptase inhibitor.

METHOD OF TREATING HUMAN IMMUNODEFICIENCY VIRUS INFECTION USING A CYCLIC **PROTEASE** INHIBITOR IN COMBINATION WITH A REVERSE TRANSCRIPTASE INHIBITOR

Abstract:
...mammal a therapeutically effective amount of a combination of: (i) at least one cyclic HIV **protease** inhibitor and (ii) at least one HIV reverse transcriptase inhibitor.
Exemplary Claim:
...the mammal a synergistically and therapeutically effective amount of: (i) at least one cyclic HIV **protease** inhibitor, and (ii) at least one HIV reverse transcriptase inhibitor selected from the group consisting of AZT, ddI, ddC, d4T, and 3TC; wherein the HIV **protease** inhibitor is selected from compounds of the Formula (I):

W<(-C(-R7)(-R7A)-(C(-R6...

...CH3)2O-; -OC(CH3)2NH- or any group that, when administered to a mammalian subject, **cleaves** to form a free dihydroxyl or diamino or hydroxyl and amino; R5a is selected from...

...substituted with 0-3 R12; or any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl, amino or sulfhydryl; R11 is selected from one or more...

Non-exemplary Claims:
2. A method of claim 1 wherein the HIV **protease** inhibitor is a compound of Formula (I) wherein: R4 and R7 are independently selected from...

...2O-; -OC(OCH3)(CH2CH2CH3)O-; or any group that, when administered to a mammalian subject, **cleaves** to form a free dihydroxyl; R5a is selected from hydrogen or fluoro; R6a is selected...

...alkylcarbonyl; C1-C6 alkoxycarbonyl; benzoyl; or any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl; R11 is selected from one or more of the following...3. A method of claim 1 wherein the HIV **protease** inhibitor is a compound of Formula (I) wherein: R4 and R7 are independently selected from...

...and R21 are independently hydrogen or any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl; R11 is selected from one or more of the following...

...4. A method of claim 3 wherein the HIV **protease** inhibitor is a compound of Formula (I) wherein: R4 and R7 are selected from benzyl...5. A method of claim 1 wherein the HIV **protease** inhibitor is a compound of the Formula (II...

...6. A method of claim 1 wherein the HIV **protease** inhibitor is a compound of Formula (IIa...7. A method of claim 6 wherein the HIV **protease** inhibitor is selected from the group of compounds consisting of: the compound of Formula (IIa...8. A method according to claim 7 wherein the HIV **protease** inhibitor is((4R-(4a, 5a, 6b, 7b))hexahydro-5,6-bis(hydroxy)-1,3-bis...

...9. A method of claim 1 wherein the HIV **protease** inhibitor is a compound of the Formula (IIaa...

...or a pharmaceutically acceptable salt or **prodrug** form thereof wherein: R4 and R7 are independently selected from: benzyl, fluorobenzyl, pyrrolylmethyl, methoxybenzyl, isobutyl...21. The method according to claim 7, wherein the HIV **protease** inhibitor is (4R-(4a, 5a, 6b, 7b))-1,2-bis((3-aminophenyl)methyl)hexahydro-5...

9/3,K,AB/13 (Item 5 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENT

Dialog Acc No: 2747898 IFI Acc No: 9619911
Document Type: C
SUBSTITUTED BICYCLIC PHOSPHORAMIDES AND DERIVATIVES THEREOF; VIRICIDES
Inventors: Smallheer Joanne M (US)
Assignee: Du Pont Merck Pharmaceutical Co Assignee Code: 25859
Patent (No,Date), Applic (No,Date)
US 5543517 19960806 US 94181117 19940113
Calculated Expiration: 20140113
Priority Applic(No,Date): US 94181117 19940113

Abstract:
This invention relates to substituted bicyclic phosphoramides and derivatives thereof, useful as retroviral **protease** inhibitors and as standards and reagents in determining the ability of a potential pharmaceutical to inhibit viral replication or HIV **protease**, to pharmaceutical compositions comprising such compounds, and to methods of using these compounds for treating viral infection.

Abstract:
This invention relates to substituted bicyclic phosphoramides and derivatives thereof, useful as retroviral **protease** inhibitors and as standards and reagents in determining the ability of a potential pharmaceutical to inhibit viral replication or HIV **protease**, to pharmaceutical compositions comprising such compounds, and to methods of using these compounds for treating...
Exemplary Claim:
...6-R6A,7-R7,7-R7A-1,3,2-DIAZAPHOSPHEPANE

or pharmaceutically acceptable salt or **prodrug** form thereof wherein: Z is O; each of R4 and R7 is independently: hydrogen, -O...

...that, when administered to a mammalian subject as part of a compound of formula (I), **cleaves** to form a compound having two free hydroxyl groups, or two free amino groups, or...that, when administered to a mammalian subject as part of a compound of formula (I), **cleaves** to form a free hydroxyl, free amino or free sulfhydryl; each of R22 and R23

...
Non-exemplary Claims:
2. A compound of claim 1, or a pharmaceutically acceptable salt or **prodrug** form thereof wherein: Each of R4 and R7 is independently: hydrogen or C1-C3 alkyl...

...that, when administered to a mammalian subject as part of a compound of formula (I), **cleaves** to form a free hydroxyl; Each of R22 and R23 is independently: hydrogen, C1-C8...

...3. A compound of claim 1, or a pharmaceutically acceptable salt or **prodrug** form thereof wherein: Both R4 and R7 are phenylmethyl; Both R4A and R7A are hydrogen...4. A compound of claim 1, or a pharmaceutically acceptable salt or **prodrug** form thereof wherein: Both R4 and R7 are phenylmethyl; Both R4A and R7A are hydrogen...

9/3,K,AB/14 (Item 6 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENT

Dialog Acc No: 2735554 IFI Acc No: 9616527
Document Type: C
METHOD FOR PREPARING N-MONOSUBSTITUTED AND N,N'-DISUBSTITUTED UNSYMMETRICAL CYCLIC UREAS; MULTISTAGE REACTION WITH ALKYLATION, DEALKYLATION AND DEPROTECTION
Inventors: Rodgers James D (US); Sun Jung-Hui (US)
Assignee: Du Pont Merck Pharmaceutical Co Assignee Code: 25859
Patent (No,Date), Applic (No,Date)
US 5532357 19960702 US 95481683 19950607
Calculated Expiration: 20150607
(Cited in 001 later patents)
Priority Applic(No,Date): US 95481683 19950607

Abstract:
The present invention relates generally to methods for the preparation of unsymmetrically substituted cyclic ureas of the formulae

D R A W I N G

which are useful as HIV **protease** inhibitors. The methods provided go through an isourea intermediate.

Abstract:
...of the formulae

D R A W I N G

which are useful as HIV **protease** inhibitors. The methods provided go through an isourea intermediate.
Exemplary Claim:
...6-DI(HO-),
7-R4-HEXAHYDRO-1,3-DIAZEPINE (VIb)

or pharmaceutically acceptable salts or **prodrug** forms thereof wherein: R4 and R7 are the same and are selected from the group...
Non-exemplary Claims:
...or pharmaceutically acceptable salts or **prodrug** forms thereof wherein: R4 and R7 are the same and are selected from the group...3 R12; a hydroxyl protecting; and any group that, when administered to a mammalian subject, **cleaves** to form a free hydroxyl group; or R5 and R6 may alternatively be taken together...
?

```
? ds

Set     Items   Description
S1      22392   PRODRUG??
S2      73467   THROMBIN
S3        140   S1 AND S2
S4          9   S3 AND PY=1998
S5          7   RD (unique items)
S6          1   S5 AND PY<=1997
S7      15536   FACTOR(W)VA OR FACTOR(W)VIIA OR FACTOR(W)IXA OR FACTOR(W)XA
              OR FACTOR XIIA
S8         84   S1 AND S7
S9          8   S8 AND PY<=1997
S10         7   RD (unique items)
S11    117513   TPA OR CVF OR C3B OR PROTEIN(W)C OR C(W)1S(W)INHIBITOR OR -
             ANTITRYPSIN OR HIRUDIN OR AT(W)II OR TFP(W)I OR PAI(W)1 OR PA-
             I(W)2 OR PAI(W)3 OR ONCOSTATIN(W)M
S12        57   S1 AND S11
S13        28   S12 AND PY<=1997
S14        26   RD (unique items)
S15         6   S14 AND PY=1996
S16        15   S14 AND PY<=1996
S17        15   RD (unique items)
? s lif or angiostatin or platelet(w)factor or TIMP? or kallikrein
          14042  LIF
           1392  ANGIOSTATIN
         289704  PLATELET
        1873863  FACTOR
           6978  PLATELET(W)FACTOR
          11133  TIMP?
          16428  KALLIKREIN
     S18  49791  LIF OR ANGIOSTATIN OR PLATELET(W)FACTOR OR TIMP? OR
                 KALLIKREIN
? s s1 and s18
          22392  S1
          49791  S18
     S19     41  S1 AND S18
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S20     31  RD (unique items)
? s s20 an dpy<=1997
>>>Term "AN" in invalid position
? s s20 and py<=1997
Processing
             31  S20
       31493495  PY<=1997
     S21     11  S20 AND PY<=1997
? t s21/3,k,ab/1-11

 21/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08646642   95405514   PMID: 7675125
  Stereoselective renal effects of the loop diuretic ozolinone in the
anesthetized dog.
  Barthelmebs M; Stephan D; Krieger J P; Grima M; Imbs J L
  Institut de Pharmacologie (URA D0589 CNRS), Faculte de Medecine,
Universite Louis Pasteur, Strasbourg, France.
  Naunyn-Schmiedeberg's archives of pharmacology (GERMANY)   Jun 1995
,  351  (6)  p660-71,  ISSN 0028-1298   Journal Code: 0326264
```

Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The renal effects of i.v. injections of (+/-)-ozolinone, its enantiomers (-)-ozolinone and (+)-ozolinone and its **prodrug** (+/-)-etozoline, were compared with those of furosemide, in pentobarbital anesthetized dogs. Renal blood flow (electromagnetic flow-meter) and glomerular filtration rate (polyfructosan clearance) were assessed on the left denervated kidney together with renin secretion and urinary electrolyte excretion. (-)-Ozolinone (15.5 mg/kg i.v.) behaves as a stereoselective loop diuretic equipotent to 20 mg/kg of furosemide and 45 mg/kg of (+/-)-ozolinone; (+)-ozolinone induced only minor salidiuretic effects. Both ozolinone enantiomers markedly increased the renal blood flow and decreased the filtration fraction, suggesting that the vosodilating effect predominates on the efferent glomerular arterioles. (-)-Ozolinone also induced an acute rise in renin secretion. The inhibition of prostaglandin synthesis (indomethacin or meclofenamate) prevented renin hypersecretion in response to (-)-ozolinone and modified its salidiuretic effects but had no effect on the vascular response. The inhibition of the **kallikrein**-kinin system by aprotinin had no effect on the overall renal response to (-)-ozolinone. The inhibition of the renin-angiotensin system by captopril decreased blood pressure, prolonged the (-)-ozolinone-induced decrease in renal vascular resistance and increased renin secretion. Our results demonstrate that the loop diuretic, ozolinone, induces stereoselective and prostaglandin-dependent renin secretion, which is involved in the regulation of intra-renal hemodynamics.

Jun **1995**,

... renal effects of i.v. injections of (+/-)-ozolinone, its enantiomers (-)-ozolinone and (+)-ozolinone and its **prodrug** (+/-)-etozoline, were compared with those of furosemide, in pentobarbital anesthetized dogs. Renal blood flow (electromagnetic...

...its salidiuretic effects but had no effect on the vascular response. The inhibition of the **kallikrein**-kinin system by aprotinin had no effect on the overall renal response to (-)-ozolinone. The...

21/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

2/21

08535070 95292870 PMID: 7774515

Ramipril. An updated review of its therapeutic use in essential hypertension and heart failure.

Frampton J E; Peters D H
Adis International Ltd, Auckland, New Zealand.

Drugs (NEW ZEALAND) Mar **1995**, 49 (3) p440-66, ISSN 0012-6667
Journal Code: 7600076
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Ramipril is a second generation angiotensin converting enzyme (ACE) inhibitor. Like enalapril, it is a **prodrug** and is hydrolysed in vivo to release the active metabolite, ramiprilat, which has a long elimination half-life, permitting once-daily administration. The antihypertensive efficacy of ramipril has been confirmed in large-scale noncomparative studies conducted in general practice as well as in more rigorously controlled clinical trials. In the former, approximately 85% of patients with mild to moderate essential hypertension have responded successfully to treatment with ramipril 2.5 or 5 mg/day, while comparative trials indicate that the antihypertensive efficacy of the drug is equivalent to that of

other established ACE inhibitors and the beta-adrenoceptor antagonist atenolol. As expected, the response rate to ramipril monotherapy is lower in patients with severe hypertension (around 40%), although the blood pressure lowering effect can be enhanced with the addition of a diuretic such as hydrochlorothiazide or piretanide. The antihypertensive efficacy of ramipril is maintained in patients with diabetes mellitus and preliminary data indicate that the drug has the beneficial effect of decreasing urinary albumin excretion in diabetic patients with nephropathy. Ramipril is superior to atenolol in causing regression of left ventricular hypertrophy, although the clinical significance of this effect per se remains to be established. The large-scale Acute Infarction Ramipril Efficacy (AIRE) study demonstrated that ramipril 5 or 10 mg/day significantly decreased the risk of all-cause mortality by 27% in patients with clinical evidence of heart failure after acute myocardial infarction, even if transient. The beneficial effect of ramipril was apparent by 30 days of treatment and appeared to be greatest in patients with more severe ventricular damage after infarction. Ramipril is well tolerated in general practice, with 5% or fewer patients discontinuing therapy because of drug intolerance. The data available suggest that ramipril shares a similar tolerability profile to that of other established ACE inhibitors. Thus, clinical data confirm ramipril as a useful alternative ACE inhibitor for the treatment of patients with mild to moderate hypertension, and indicate a beneficial effect of the drug in patients with clinical evidence of heart failure after acute myocardial infarction. It is also reasonable to assume that ramipril will be of value in the treatment of patients with more established heart failure or asymptomatic left ventricular dysfunction.

Mar **1995**,

Ramipril is a second generation angiotensin converting enzyme (ACE) inhibitor. Like enalapril, it is a **prodrug** and is hydrolysed in vivo to release the active metabolite, ramiprilat, which has a long...

...; CO; Hypertension--metabolism--ME; Hypertrophy, Left Ventricular --complications--CO; Hypertrophy, Left Ventricular--drug therapy--DT; **Kallikrein**-Kinin System--drug effects--DE; Kidney--drug effects--DE; Myocardial Infarction--complications--CO; Myocardial Infarction...

21/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06646217 90344075 PMID: 2383374

Ethnic differences in the renal sodium dopamine relationship. A possible explanation for regional variations in the prevalence of hypertension?

Lee M R; Critchley J A; Gordon C J; Makarananda K; Sriwatanakul K; Balali-Mood M; Boye G L

University Department of Clinical Pharmacology, Royal Infirmary, Edinburgh, Scotland.

American journal of hypertension : journal of the American Society of Hypertension (UNITED STATES) Jun **1990**, 3 (6 Pt 2) p100S-103S, ISSN 0895-7061 Journal Code: 8803676

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Over the last three years we have carried out studies on the urine output of both sodium and dopamine in five different ethnic groups: whites, Ghanaians, Zimbabweans, Iranians and Thais. Sodium was measured by ion specific electrode and dopamine by HPLC with electrochemical detection (using epinine as an internal standard). In several groups salt loading studies were also carried out. The five ethnic groups differed substantially with regard to the correlation between their urinary sodium and dopamine outputs. Three groups (whites, Thais and Zimbabweans) showed a strong positive correlation (P less than .001) and this may reflect their

traditionally salt rich diet. In two groups (Ghanaians and Iranians) there was no correlation and this may reflect a salt scarce environment. Taken together with our previously reported studies showing that normotensive Ghanaians do not mobilize dopamine on salt loading, this would suggest that certain ethnic groups are predisposed to develop hypertension on salt loading--that is, they are 'salt sensitive.' This genetic trait may have passed from the West Coast of Africa, with the slaves, to America and the Caribbean. Other workers have reported deficiencies in vasodilator systems in the American black, such as dopamine, **kallikrein** and the renal prostaglandins. These defects may lead to the nosologic entity of 'low renin' hypertension, well described in American blacks, and could open up avenues of therapy based either on DA1 activators (such as fenoldopam) or on renal **prodrugs** (such as gludopa).

Jun **1990**,
...Other workers have reported deficiencies in vasodilator systems in the American black, such as dopamine, **kallikrein** and the renal prostaglandins. These defects may lead to the nosologic entity of 'low renin...
... up avenues of therapy based either on DA1 activators (such as fenoldopam) or on renal **prodrugs** (such as gludopa).

21/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06468368 90177313 PMID: 2408245
[Clinical pharmacology of ACE inhibitors]
Klinische Pharmakologie der ACE-Hemmer.
Hitzenberger G
I. Medizinische Universitatsklinik, Abteilung fur Klinische Pharmakologie, Wien.
Wiener medizinische Wochenschrift (1946) (AUSTRIA) Jan 31 **1990**, 140 (1-2) p12-8, ISSN 0043-5341 Journal Code: 8708475
Document type: Journal Article; Review; Review, Tutorial ; English Abstract
Languages: GERMAN
Main Citation Owner: NLM
Record type: Completed
The main effect of the ACE-inhibitors is a reduction of the peripheral resistance and according to that an increase of blood-flow to the organs. Direct effects on the heart are of minor importance. The exact mechanism of action is not fully understood; the main role plays obviously the inhibition of the angiotensin converting enzyme itself; in addition may be effects on the **kallikrein** -bradykinin-prostaglandin-system are of importance. The pharmacodynamical effects depend on the plasma-concentration and therefore on the pharmacokinetics. These are different with Captopril and Enalapril: Captopril acts directly whereas Enalapril is a "**prodrug**". With the nowadays used doses Captopril and Enalapril are widely free of side-effects. With the exception of patients with negative sodium-balance (salt-poor diet and/or treatment with diuretics) or patients with renal insufficiency (sometimes increase of creatinine and potassium). In the first group of patients the first dose of ACE-inhibitors should be administered in the evening before going to bed, in the second group creatinine and potassium must be checked 5 to 7 days after an initiation of treatment.

Jan 31 **1990**,
... the inhibition of the angiotensin converting enzyme itself; in addition may be effects on the **kallikrein** -bradykinin-prostaglandin-system are of importance. The pharmacodynamical effects depend on the plasma-concentration and...

... pharmacokinetics. These are different with Captopril and Enalapril: Captopril acts directly whereas Enalapril is a "**prodrug**". With the nowadays used doses Captopril and Enalapril are widely free of side-effects. With...

21/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05092144 86163820 PMID: 3007062
Enalapril: a new angiotensin converting enzyme inhibitor.
Cleary J D; Taylor J W
Drug intelligence & clinical pharmacy (UNITED STATES) Mar **1986**, 20 (3) p177-86, ISSN 0012-6578 Journal Code: 0212457
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Enalapril maleate is a new angiotensin converting enzyme inhibitor marketed in the U.S. by Merck Sharp and Dohme. It has been demonstrated to actively interfere with the renin-angiotensin-aldosterone system. This is reflected by both hemodynamic (decreased blood pressure) and humoral (increased plasma renin, angiotensin I, and decreased angiotensin II) responses to enalapril therapy. Activity in the **kallikrein**-bradykinin system is still controversial. Enalapril maleate is a **prodrug** which is quickly absorbed, hydrolyzed by the liver to the active metabolite enalaprilic acid, and excreted 33 percent in the bile and 61 percent in the urine. The therapeutic dosage range is 10-40 mg/d, maximum of 40 mg, given once or twice daily. The onset and duration of action are dose related. Vertigo and headache have been the most commonly reported side effects. Clinical comparison of enalapril to hydrochlorothiazide, beta-adrenergic blockers, and captopril find it efficacious in the treatment of essential hypertension. Efficacy in treating congestive heart failure and hypertension secondary to renal artery stenosis has also been demonstrated for both angiotensin converting enzyme inhibitors. The overall efficacy and safety of enalapril and captopril appear equivalent when used at low doses in patients with uncomplicated hypertension.

Mar **1986**,
... plasma renin, angiotensin I, and decreased angiotensin II) responses to enalapril therapy. Activity in the **kallikrein**-bradykinin system is still controversial. Enalapril maleate is a **prodrug** which is quickly absorbed, hydrolyzed by the liver to the active metabolite enalaprilic acid, and...

21/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03911316 82185350 PMID: 6804154
Dopamine and the kidney.
Lee M R
Clinical science (London, England : 1979) (ENGLAND) May **1982**, 62 (5) p439-48, ISSN 0143-5221 Journal Code: 7905731
Document type: Journal Article; Review
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

It would seem established beyond peradventure that dopamine is formed in the kidney from circulating L-dopa. The likely site would appear to be the renal tubular cells but the contribution of the renal dopaminergic nerves needs further evaluation. Moreover it is probably that dopamine formed

within the kidney acts there on specific receptors. This results in vasodilatation of renal blood vessels, by action on vascular receptors, and natriuresis, by an effect on tubular sodium transport mechanisms. Dopamine may form an integral part of the renal natriuretic cascade by, in its turn, evoking both the **kallikrein**-bradykinin system and the production of renal prostaglandins. Specific activation of the renal dopaminergic system by the administration of suitable agonists or renal **prodrugs** may prove possible in the future. Abnormalities in the renal production of dopamine may be important in several hypertensive and oedematous disorders. Further work will be required to establish a possible role for dopamine in these conditions and to determine whether they will benefit from treatment with suitable dopamine agonists. Dopamine, once regarded as of little importance outside the central nervous system, has certainly come to occupy a central place in renal salt handling. The ratio of dopamine production in the kidney to that for renin may be of pivotal importance in the control of systemic arterial pressure.

May **1982**,

... an integral part of the renal natriuretic cascade by, in its turn, evoking both the **kallikrein**-bradykinin system and the production of renal prostaglandins. Specific activation of the renal dopaminergic system by the administration of suitable agonists or renal **prodrugs** may prove possible in the future. Abnormalities in the renal production of dopamine may be...

21/3,K,AB/7 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06239794 Genuine Article#: YD713 Number of References: 48
Title: Potentiation of the actions of bradykinin by angiotensin I-converting enzyme inhibitors - The role of expressed human bradykinin B-2 receptors and angiotensin I-converting enzyme in CHO cells (ABSTRACT AVAILABLE)
Author(s): Minshall RD; Tan FL; Nakamura F; Rabito SF; Becker RP; Marcic B; Erdos EG (REPRINT)
Corporate Source: UNIV ILLINOIS,COLL MED, DEPT PHARMACOL, M-C 868, 835 S WOLCOTT AVE/CHICAGO//IL/60612 (REPRINT); UNIV ILLINOIS,COLL MED, DEPT PHARMACOL/CHICAGO//IL/60612; UNIV ILLINOIS,COLL MED, DEPT ANESTHESIOL/CHICAGO//IL/60612; UNIV ILLINOIS,COLL MED, DEPT ANAT & CELL BIOL/CHICAGO//IL/60612; COOK CTY HOSP,DEPT ANESTHESIOL/CHICAGO//IL/60612
Journal: CIRCULATION RESEARCH, **1997**, V81, N5 (NOV), P848-856
ISSN: 0009-7330 Publication date: 19971100
Publisher: AMER HEART ASSOC, 7272 GREENVILLE AVENUE, DALLAS, TX 75231-4596
Language: English Document Type: ARTICLE
Abstract: Part of the beneficial effects of angiotensin I-converting enzyme (ACE) inhibitors are due to augmenting the actions of bradykinin (BK). We studied this effect of enalaprilat on the binding of [H-3]BK to Chinese hamster ovary (CHO) cells stably transfected to express the human BK B-2 receptor alone (CHO-3B) or in combination with ACE (CHO-15AB). In CHO-15AB cells, enalaprilat (1 mu mol/L) increased the total number of low-affinity [H-3]BK binding sites on the cells at 37 degrees C, but not at 4 degrees C, from 18.4+/-4.3 to 40.3+/-11.9 fmol/10(6) cells (P<.05; K-d, 2.3+/-0.8 and 5.9+/-1.3 nmol/L; n=4). Enalaprilat preserved a portion of the receptors in high-affinity conformation (K-d, 0.17+/-0.08 nmol/L; 8.1+/-0.9 fmol/10(6) cells). Enalaprilat decreased the IC50 of [Hyp(3)-Tyr(Me)(8)]BK, the BK analogue more resistant to ACE, from 3.2+/-0.8 to 0.41+/-0.16 nmol/L (P<.05, n=3). The biphasic displacement curve of the binding of [H-3]BK also suggested the presence of high-affinity BK binding sites. Enalaprilat (5 nmol to 1 mu mol/L) potentiated the release of [H-3]arachidonic acid and the liberation of inositol

1,4,5-trisphosphate (IP3) induced by BK and [Hyp(3)-Tyr(Me)(8)]BK. Moreover, enalaprilat (1 mu mol/L) completely and immediately restored the response of the B-2 receptor, desensitized by the agonist (1 mu mol/L [Hyp(3)-Tyr(Me)(8)]BK); this effect was blocked by the antagonist, HOE 140. Finally, enalaprilat, but not the **prodrug** enalapril, decreased internalization of the receptor from 70+/-9% to 45+/-9% (P<.05, n=7). In CHO-3B cells, enalaprilat was ineffective. ACE inhibitors in the presence of both the B-2 receptor and ACE enhance BK binding, protect high-affinity receptors, block receptor desensitization, and decrease internalization, thereby potentiating BK beyond blocking its hydrolysis.

, **1997**
...Abstract: BK); this effect was blocked by the antagonist, HOE 140. Finally, enalaprilat, but not the **prodrug** enalapril, decreased internalization of the receptor from 70+/-9% to 45+/-9% (P<.05, n...
...Research Fronts: LEFT-VENTRICULAR DYSFUNCTION; EARLY ENALAPRIL THERAPY)
95-2633 001 (NONPEPTIDE BRADYKININ B-2 RECEPTOR ANTAGONISTS; **KALLIKREIN** GENE FAMILY EXPRESSION IN THE RAT OVARY; GUINEA-PIG ILEUM MEMBRANES)
95-3190 001 (INCREASED...

21/3,K,AB/8 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

2/21

04527860 Genuine Article#: TK374 Number of References: 347
Title: GENE-THERAPY FOR BRAIN-TUMORS (Abstract Available)
Author(s): KRAMM CM; SENAESTEVES M; BARNETT FH; RAINOV NG; SCHUBACK DE; YU JS; PECHAN PA; PAULUS W; CHIOCCA EA; BREAKEFIELD XO
Corporate Source: MASSACHUSETTS GEN HOSP EAST,MOLEC NEUROGENET UNIT,13 ST,BLDG 149/BOSTON//MA/02129; MASSACHUSETTS GEN HOSP,CTR NEUROSCI,MOLEC NEUROGENET UNIT/BOSTON//MA/02129; HARVARD UNIV,SCH MED,DEPT NEUROL/BOSTON//MA/02115; UNIV DUSSELDORF,CHILDRENS HOSP/W-4000 DUSSELDORF//GERMANY/; BRIGHAM & WOMENS HOSP,CHILDRENS HOSP,NEUROL SERV/BOSTON//MA/02115; UNIV HALLE WITTENBERG,DEPT NEUROSURG/HALLE//GERMANY/; MASSACHUSETTS GEN HOSP,NEUROL SERV/BOSTON//MA/02114; UNIV ZURICH HOSP,DEPT PATHOL,INST NEUROPATHOL/CH-8091 ZURICH//SWITZERLAND/
Journal: BRAIN PATHOLOGY, **1995**, V5, N4 (OCT), P345-381
ISSN: 1015-6305
Language: ENGLISH Document Type: REVIEW
Abstract: Gene therapy has opened new doors for treatment of neoplastic diseases. This new approach seems very attractive, especially for glioblastomas, since treatment of these brain tumors has failed using conventional therapy regimens. Many different modes of gene therapy for brain tumors have been tested in culture and in vivo. Many of these approaches are based on previously established anti-neoplastic principles, like **prodrug** activating enzymes, inhibition of tumor neovascularization, and enhancement of the normally weak anti-tumor immune response. Delivery of genes to tumor cells has been mediated by a number of viral and synthetic vectors. The most widely used paradigm is based on the activation of ganciclovir to a cytotoxic compound by a viral enzyme, thymidine kinase, which is expressed by tumor cells, after the gene has been introduced by a retroviral vector. This paradigm has proven to be a potent therapy with minimal side effects in several rodent brain tumor models, and has proceeded to phase 1 clinical trials. In this review, current gene therapy strategies and vector systems for treatment of brain tumors will be described and discussed in light of further developments needed to make this new treatment modality clinically efficacious.

, **1995**

...Abstract: in vivo. Many of these approaches are based on previously established anti-neoplastic principles, like **prodrug** activating enzymes, inhibition of tumor neovascularization, and enhancement of the normally weak anti-tumor immune...
...Identifiers--REGION-4; THYMIDINE KINASE GENE; ANTI-SENSE RNA; JUNCTIONAL PROTEIN CONNEXIN-43; PACKAGING CELL-LINE; **PLATELET FACTOR** -IV

21/3,K,AB/9 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01561585 Genuine Article#: HH156 Number of References: 15
Title: LINEAR TRIPEPTIDES AND TETRAPEPTIDES ACTING AS **PRODRUGS** (Abstract Available)
Author(s): KASAFIREK E; STURC A; ROUBALOVA A
Corporate Source: RES INST PHARM & BIOCHEM/CS-13060 PRAGUE 3//CZECHOSLOVAKIA/
Journal: COLLECTION OF CZECHOSLOVAK CHEMICAL COMMUNICATIONS, **1992**, V 57, N1 (JAN), P179-187
Language: ENGLISH Document Type: ARTICLE
Abstract: Tri- and tetrapeptides with C-terminal 1-amino-1-cycloalkanecarboxylic acid of the general formula X-Ala-Y-OR, where X is Ala, Leu, Phe, Ac-Tyr, Gly-Pro, Ac-Leu-Lys or Ac-Leu-Arg, Y is Acb, Acp or Ach, and R is methyl or ethyl, have been prepared. These peptides containing a rationally chosen N-substituent are cleaved with a suitable enzyme (leucinaminopeptidase, alaninaminopeptidase, chymotrypsin, plasmin or **kallikrein**) in an aqueous medium. The arising C-terminal dipeptide ester undergoes spontaneous cyclization to give biologically active spirocyclic dipeptide. The prepared short peptides exhibit all functional features of **prodrugs**.

Title: LINEAR TRIPEPTIDES AND TETRAPEPTIDES ACTING AS **PRODRUGS** , **1992**
...Abstract: rationally chosen N-substituent are cleaved with a suitable enzyme (leucinaminopeptidase, alaninaminopeptidase, chymotrypsin, plasmin or **kallikrein**) in an aqueous medium. The arising C-terminal dipeptide ester undergoes spontaneous cyclization to give biologically active spirocyclic dipeptide. The prepared short peptides exhibit all functional features of **prodrugs**.

21/3,K,AB/10 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3327555 IFI Acc No: 0015882
Document Type: C
GENE THERAPY FOR SOLID TUMORS USING ADENOVIRAL VECTORS COMPRISING SUICIDE GENES AND CYTOKINE GENES; TOGETHER WITH **PRODRUG**
Inventors: Chen Shu-Hsia (US); Woo Savio L C (US)
Assignee: Baylor College of Medicine
Assignee Code: 06345
Publication (No,Date), Applic (No,Date):
US 6066624 20000523 US 96600942 19960215
Publication Kind: A
Calculated Expiration: 20170523
(Cited in 001 later patents)
Cont.-in-part Pub(No),Applic(No,Date): US 5631236 US 93112745 19930826
PCT Pub(No,Date),Applic(No,Date): WO 955835 **19950302** WO 94US9784 19940825
Section 371: 19960215

Section 102(e):19960215
Priority Applic(No,Date): US 96600942 19960215; US 93112745 19930826

Abstract: The present invention provides a novel method of treating localized solid tumors and papillomas in an individual, as well as metastatic carcinomas. The method comprises delivering a suicide gene, by way of a recombinant adenoviral vector or other DNA transport system, into the tumor, papilloma or wart of an individual. Subsequently, a **prodrug**, such as the drug gaciclovir(tm), is administered to the individual. Additionally, the present invention provides a method for treating solid tumors, papillomas, warts and metastatic carcinomas, said method comprising introducing both a suicide gene and one or more cytokine genes into the tumor, papilloma or wart of an individual, and subsequently administering a **prodrug** to the individual. The methods of the present invention may be used to treat several different types of cancers and papillomas, including colon carcinoma, prostate cancer, breast cancer, lung cancer, melanoma, hepatoma, brain lymphoma and head and neck cancer.

...TOGETHER WITH **PRODRUG**
...PCT Pub(No,Date),Applic(No,Date): **19950302**

Abstract: ...other DNA transport system, into the tumor, papilloma or wart of an individual. Subsequently, a **prodrug**, such as the drug gaciclovir(tm), is administered to the individual. Additionally, the present invention...
...cytokine genes into the tumor, papilloma or wart of an individual, and subsequently administering a **prodrug** to the individual. The methods of the present invention may be used to treat several...

Exemplary Claim: ...tumor expresses said suicide gene and said one or more cytokine genes; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene.
Non-exemplary Claims: ...suicide gene sequence to be expressed is thymidine kinase of herpes simplex virus and the **prodrug** is ganciclovir, acyclovir, FIAU or their derivatives...

...1, wherein the suicide gene sequence to be expressed is bacteria cytosine deaminase and the **prodrug** is 5-fluorocytosine or its derivatives...

...the suicide gene sequence to be expressed is varicella zoster virus thymidine kinase and the **prodrug** is 6-methoxypurine arabinoside or its derivatives...

...10. The method of claim 1, wherein said **prodrug** is selected from the group consisting of ganciclovir, acyclovir, 1-5-iodouracil FIAU, 5-fluorocytosine...linked to a promoter and wherein said tumor expresses the cytokine gene; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene...

...suicide gene sequence to be expressed is thymidine kinase of herpes simplex virus and the **prodrug** is ganciclovir, acyclovir, FIAU or their derivatives...

...14, wherein the suicide gene sequence to be expressed is bacterial cytosine deaminase and the **prodrug** is 5-fluorocytosine or its derivatives...

...the suicide gene sequence to be expressed is varicella zoster virus thymidine kinase and the **prodrug** is 6-methoxypurine arabinoside or its derivatives...of a prostate specific antigen promoter, a gp91-phox

gene promoter and a prostate-specific **kallikrein** promoter...

...36. The method of claim 14, wherein said **prodrug** is selected from the group consisting of ganciclovir, acyclovir, 1-5-iodouracil FIAU, 5-fluorocytosine...wherein said tumor expresses said suicide gene and said IL-2 gene; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene...
...linked to a promoter and wherein said tumor expresses the cytokine gene; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene...

21/3,K,AB/11 (Item 2 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 2530114 IFI Acc No: 9422741
Document Type: C
TOCOTRIENOLS IN THE TREATMENT OF HYPERCHOLESTEROLEMIA, HYPERLIPIDEMIA AND THROMBOEMBOLIC DISORDERS
Inventors: Parker Rex (US); Pearce Bradley C (US); Qureshi Asaf A (US); Wright John J (US)
Assignee: Bristol-Myers Squibb Co
Assignee Code: 22921
Publication (No,Date), Applic (No,Date):
US 5348974 **19940920** US 9315778 19930210
Publication Kind: A
Calculated Expiration: 20110920
(Cited in 010 later patents)
Continuation Pub(No),Applic(No,Date): US 5217992 US 90583907 19900917
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US 89416910 19891004
Priority Applic(No,Date): US 9315778 19930210; US 90583907 19900917; US 89416910 19891004

Abstract: This invention relates to the use of tocotrienol, gammatocotrienol and delta-tocotrienol in reducing hypercholesterolemia, hyperlipidemia and thromboembolic disorders in mammals. The isolation of these tocotrienols from natural sources and their chemical synthesis is disclosed. The present invention also relates to **prodrugs** and pharmaceutical compositions of gamma-tocotrienol, delta-tocotrienol and tocotrienol and uses thereof.

Publication (No,Date), Applic (No,Date):
...**19940920**

Abstract: ...from natural sources and their chemical synthesis is disclosed. The present invention also relates to **prodrugs** and pharmaceutical compositions of gamma-tocotrienol, delta-tocotrienol and tocotrienol and uses thereof.
Non-exemplary Claims: 2. A method of reducing, in a bird or mammal, thromboxane A2 and **platelet factor** 4 levels providing antithrombotic effectiveness which comprises administering to such bird or mammal a safe...

...which comprises administering to such bird or mammal a safe and effective amount of the **prodrug** of gamma-tocotrienol, delta-tocotrienol or tocotrienol, or mixtures thereof...

...4. A method of reducing, in a bird or a mammal, thromboxane A2 and

platelet factor 4 levels providing antithrombotic effectiveness which comprises administering to such bird or mammal a safe and effective amount of the **prodrug** of gamma-tocotrienol, delta-tocotrienol or tocotrienol, or mixtures thereof...

?

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      ---  -----  -----------
? s prodrug??
      S1   22392  PRODRUG??
? s thrombin
      S2   73467  THROMBIN
? s s1 and s2
           22392  S1
           73467  S2
      S3     140  S1 AND S2
? s s3 and py=1998
             140  S3
         2108721  PY=1998
      S4       9  S3 AND PY=1998
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>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S5       7  RD (unique items)
? s s5 and py<=1997
Processing
Processing
               7  S5
        31493495  PY<=1997
      S6       1  S5 AND PY<=1997
? t s6/3,k,ab/1

 6/3,K,AB/1     (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent
(c) 2003 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 2974824  IFI Acc No: 9813720
Document Type: C
INHIBITORS OF PLATELET AGGREGATION; ANTITHROMBIN AND ANTICOAGULANTS
Inventors: Cox David (GB); Ingall Anthony (GB); Willis Paul (GB)
Assignee: Astra Pharmaceuticals Ltd GB
Assignee Code: 42547  Document Type: REASSIGNED
Publication (No,Date), Applic (No,Date):
US 5747496     19980505 US 96737005    19961031
Publication Kind:  A
Calculated Expiration: 20160704
(Cited in 002 later patents)
PCT Pub(No,Date),Applic(No,Date): WO 973084       19970130  WO
96SE911     19960704
    Section 371:  19961031
    Section 102(e):19961031
Priority Applic(No,Date): GB 9514074     19950711; GB 9520311     19951005;
GB 9522837     19951108

Abstract: Compounds of the formula (I)

                          D R A W I N G

     wherein B is O or CH2; X is selected from NR1R2, SR1, and C1-C7 alkyl;
Y is selected SR1, NR1R2, and C1-C7 alkyl; R1 and R2 is each and
independently selected from H, or C1-C7 alkyl optionally substituted upon
or within the alkyl chain by one or more of O, S, N or halogen; R3 and R4
are both H, or R3 and R4 together form a bond; A is COOH, C(O)NH(CH2)pCOOH,
C(O)N((CH2)qCOOH)2, C(O)NHCH(COOH)(CH2)rCOOH, or 5-tetrazolyl, wherein p, q
and r is each and independently 1, 2 or 3; as well as pharmaceutically
acceptable salts and prodrugs thereof, pharmaceutical compositions
comprising the novel compounds and use of the compounds in therapy. Also
within the scope of the invention are novel intermediates to the novel
compounds. The novel compounds are in particular useful in the prevention
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of platelet aggregation.

...**ANTITHROMBIN** AND ANTICOAGULANTS
Publication (No,Date), Applic (No,Date):
...**19980505**
...PCT Pub(No,Date),Applic(No,Date): **19970130**

Abstract: ...is each and independently 1, 2 or 3; as well as pharmaceutically acceptable salts and **prodrugs** thereof, pharmaceutical compositions comprising the novel compounds and use of the compounds in therapy. Also...

Exemplary Claim: ...is each and independently 1, 2 or 3; as well as pharmaceutically acceptable salts and **prodrugs** thereof.
Non-exemplary Claims: ...to 4 carbon atoms in the alkyl portion; as well as pharmaceutically acceptable salts and **prodrugs** thereof.
?

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? s factor(w)Va or factor(w)VIIa or factor(w)IXa or factor(w)Xa or factor XIIa
         1873863  FACTOR
           23514  VA
            1975  FACTOR(W)VA
         1873863  FACTOR
            6155  VIIA
            4243  FACTOR(W)VIIA
         1873863  FACTOR
            1983  IXA
            1449  FACTOR(W)IXA
         1873863  FACTOR
           14343  XA
           10184  FACTOR(W)XA
             391  FACTOR XIIA
      S7   15536  FACTOR(W)VA OR FACTOR(W)VIIA OR FACTOR(W)IXA OR
                  FACTOR(W)XA OR FACTOR XIIA
? ds

Set     Items   Description
S1      22392   PRODRUG??
S2      73467   THROMBIN
S3        140   S1 AND S2
S4          9   S3 AND PY=1998
S5          7   RD (unique items)
S6          1   S5 AND PY<=1997
S7      15536   FACTOR(W)VA OR FACTOR(W)VIIA OR FACTOR(W)IXA OR FACTOR(W)XA
              OR FACTOR XIIA
? s s1 and s7
           22392  S1
           15536  S7
      S8      84  S1 AND S7
? s s8 and py<=1997
Processing
              84  S8
        31493495  PY<=1997
      S9       8  S8 AND PY<=1997
? rd
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>>>Records from unsupported files will be retained in the RD set.
...completed examining records
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? t s10/3,k,ab/1-7

 10/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08609031   95369143   PMID: 7641602
  Novel antithrombotic drugs in development.
  Verstraete M; Zoldhelyi P
  Center for Molecular and Vascular Biology, University of Leuven, Belgium.
  Drugs (NEW ZEALAND)   Jun 1995,  49  (6)  p856-84,  ISSN 0012-6667
Journal Code: 7600076
  Document type: Journal Article; Review; Review, Academic
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  Platelet activation plays a critical role in thromboembolic disorders,
and aspirin remains a keystone in preventive strategies. This remarkable
efficacy is rather unexpected, as aspirin selectively inhibits platelet
aggregation mediated through activation of the arachidonic-thromboxane
pathway, but not platelet aggregation induced by adenosine diphosphate
(ADP), collagen and low levels of thrombin. This apparent paradox has
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stimulated investigations on the effect of aspirin on eicosanoid-independent effects of aspirin on cellular signalling. It has also fostered the search for antiplatelet drugs inhibiting platelet aggregation at other levels than the acetylation of platelet cyclo-oxygenase, such as thromboxane synthase inhibitors and thromboxane receptor antagonists. The final step of all platelet agonists is the functional expression of glycoprotein (GP) IIb/IIIa on the platelet surface, which ligates fibrinogen to link platelets together as part of the aggregation process. Agents that interact between GPIIb/IIIa and fibrinogen have been developed, which block GPIIb/IIIa, such as monoclonal antibodies to GPIIb/IIIa, and natural and synthetic peptides (disintegrins) containing the Arg-Gly-Asp (RGD) recognition sequence in fibrinogen and other adhesion macromolecules. Also, some non-peptide RGD mimetics have been developed which are orally active **prodrugs**. Stable analogues of prostacyclin, some of which are orally active, are also available. Thrombin has a pivotal role in both platelet activation and fibrin generation. In addition to natural and recombinant human antithrombin III, direct antithrombin III-independent thrombin inhibitors have been developed as recombinant hirudin, hirulog, argatroban, boroarginine derivatives and single stranded DNA oligonucleotides (aptanes). Direct thrombin inhibitors do not affect thrombin generation and may leave some 'escaping' thrombin molecules unaffected. Inhibition of **factor Xa** can prevent thrombin generation and disrupt the thrombin feedback loop that amplifies thrombin production.

Jun **1995**,
...adhesion macromolecules. Also, some non-peptide RGD mimetics have been developed which are orally active **prodrugs** . Stable analogues of prostacyclin, some of which are orally active, are also available. Thrombin has...

...do not affect thrombin generation and may leave some 'escaping' thrombin molecules unaffected. Inhibition of **factor Xa** can prevent thrombin generation and disrupt the thrombin feedback loop that amplifies thrombin production.

10/3,K,AB/2 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00678211 Genuine Article#: EM456 Number of References: 40
Title: IDENTIFICATION AND CHARACTERIZATION OF A PHOSPHOLIPID-BINDING SITE OF BOVINE **FACTOR-VA**
Author(s): KALAFATIS M; JENNY RJ; MANN KG
Corporate Source: UNIV VERMONT,COLL MED,DEPT BIOCHEM,HLTH SCI COMPLEX/BURLINGTON//VT/05405; UNIV VERMONT,COLL MED,DEPT BIOCHEM,HLTH SCI COMPLEX/BURLINGTON//VT/05405
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1990**, V265, N35, P 21580-21589
Language: ENGLISH Document Type: ARTICLE

Title: IDENTIFICATION AND CHARACTERIZATION OF A PHOSPHOLIPID-BINDING SITE OF BOVINE **FACTOR-VA**
, **1990**
Research Fronts: 88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG** -BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)
88-1470 001 (AMINO-ACID SEQUENCE; AVIAN...

10/3,K,AB/3 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00560929 Genuine Article#: EC362 Number of References: 38
Title: PROTEOLYTIC ACTIVATION OF HUMAN FACTOR-IX AND FACTOR-X BY RECOMBINANT HUMAN **FACTOR-VIIA** - EFFECTS OF CALCIUM, PHOSPHOLIPIDS, AND TISSUE FACTOR
Author(s): KOMIYAMA Y; PEDERSEN AH; KISIEL W
Corporate Source: UNIV NEW MEXICO,SCH MED,DEPT PATHOL,BLOOD SYST RES FDN LAB/ALBUQUERQUE//NM/87131; UNIV NEW MEXICO,SCH MED,DEPT PATHOL,BLOOD SYST RES FDN LAB/ALBUQUERQUE//NM/87131; NOVO IND AS,NOVO RES INST/DK-2800 BAGSVAERD//DENMARK/
Journal: BIOCHEMISTRY, **1990**, V29, N40, P9418-9425
Language: ENGLISH Document Type: ARTICLE

Title: PROTEOLYTIC ACTIVATION OF HUMAN FACTOR-IX AND FACTOR-X BY RECOMBINANT HUMAN **FACTOR-VIIA** - EFFECTS OF CALCIUM, PHOSPHOLIPIDS, AND TISSUE FACTOR
, **1990**
Research Fronts: 88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG** -BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)

10/3,K,AB/4 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00544944 Genuine Article#: EA857 Number of References: 40
Title: FUNCTIONAL-CHARACTERIZATION OF HUMAN PLATELET-RELEASED FACTOR-V AND ITS ACTIVATION BY **FACTOR-XA** AND THROMBIN
Author(s): MONKOVIC DD; TRACY PB
Corporate Source: UNIV VERMONT,DEPT BIOCHEM,C-201 GIVEN BLDG/BURLINGTON//VT/05405; UNIV VERMONT,DEPT BIOCHEM,C-201 GIVEN BLDG/BURLINGTON//VT/05405; UNIV VERMONT,DEPT MED/BURLINGTON//VT/05405
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1990**, V265, N28, P 17132-17140
Language: ENGLISH Document Type: ARTICLE

Title: FUNCTIONAL-CHARACTERIZATION OF HUMAN PLATELET-RELEASED FACTOR-V AND ITS ACTIVATION BY **FACTOR-XA** AND THROMBIN
, **1990**
Research Fronts: 88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG** -BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)
88-1153 001 (RABBIT PLATELETS; IMMUNOCYTOCHEMICAL LOCALIZATION...

10/3,K,AB/5 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00205307 Genuine Article#: CY587 Number of References: 33
Title: THE ACTIVE-SITE OF MEMBRANE-BOUND MEIZOTHROMBIN - A FLUORESCENCE DETERMINATION OF ITS DISTANCE FROM THE PHOSPHOLIPID SURFACE AND ITS CONFORMATIONAL SENSITIVITY TO CALCIUM AND **FACTOR-VA**
Author(s): ARMSTRONG SA; HUSTEN EJ; ESMON CT; JOHNSON AE
Corporate Source: UNIV OKLAHOMA,DEPT CHEM & BIOCHEM/NORMAN//OK/73019; UNIV OKLAHOMA,DEPT CHEM & BIOCHEM/NORMAN//OK/73019; OKLAHOMA MED RES FDN,CARDIOVASC BIOL RES PROGRAM/OKLAHOMA CITY//OK/73104; HOWARD HUGHES MED INST/OKLAHOMA CITY//OK/73104; UNIV OKLAHOMA,HLTH SCI CTR,DEPT PATHOL/OKLAHOMACITY//OK/73190; UNIV OKLAHOMA,HLTH SCI CTR,DEPT BIOCHEM & MOLECBIOL/OKLAHOMA CITY//OK/73190
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1990**, V265, N11, P6210-6218
Language: ENGLISH Document Type: ARTICLE

...Title: DETERMINATION OF ITS DISTANCE FROM THE PHOSPHOLIPID SURFACE AND ITS CONFORMATIONAL SENSITIVITY TO CALCIUM AND **FACTOR-VA**
, **1990**

Research Fronts: 88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG** -BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)

10/3,K,AB/6 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00084985 Genuine Article#: CM453 Number of References: 65
Title: ACTIVATION OF HUMAN FACTOR-V BY **FACTOR-XA** AND THROMBIN
Author(s): MONKOVIC DD; TRACY PB
Corporate Source: UNIV VERMONT,DEPT MED,C201/BURLINGTON//VT/05405; UNIV VERMONT,DEPT MED,C201/BURLINGTON//VT/05405; UNIV VERMONT,DEPT BIOCHEM/BURLINGTON//VT/05405
Journal: BIOCHEMISTRY, **1990**, V29, N5, P1118-1128
Language: ENGLISH Document Type: ARTICLE

Title: ACTIVATION OF HUMAN FACTOR-V BY **FACTOR-XA** AND THROMBIN
, **1990**
...Research Fronts: AXOLININ LOCALIZATION; CALCIUM-ACTIVATED PROTEIN-KINASE; GLUTATHIONE S-TRANSFERASE)
88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG**-BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)
88-1104 001 (LOW-MOLECULAR WEIGHT HEPARIN...

10/3,K,AB/7 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3525913 IFI Acc No: 0122257
Document Type: C
INHIBITORS OF **FACTOR XA**; ADMINISTERING ARGININE-CONTAINING OLIGOPEPTIDE AS ANTICOAGULANT; CARDIOVASCULAR DISORDERS
Inventors: Laibelman Alan M (US); Marlowe Charles K (US); Scarborough Robert M (US); Sinha Uma (US); Zhu Bing-Yan (US)
Assignee: COR Therapeutics Inc
Assignee Code: 32383
Publication (No,Date), Applic (No,Date):
US 6245743 20010612 US 9877001 19980515
Publication Kind: B
Calculated Expiration: 20160605
PCT Pub(No,Date),Applic(No,Date): WO 9640743 **19961219** WO 96US9285 19960605
Section 371: 19980515
Section 102(e):19980515
Priority Applic(No,Date): US 9877001 19980515

Abstract: Novel compounds, their salts and compositions related thereto having activity against mammalian **factor Xa** are disclosed. The compounds are useful in vitro or in vivo for preventing or treating coagulation disorders.

INHIBITORS OF **FACTOR XA**;
...PCT Pub(No,Date),Applic(No,Date): **19961219**

Abstract: Novel compounds, their salts and compositions related thereto having activity against mammalian **factor Xa** are disclosed. The compounds are useful in vitro or in vivo for preventing or treating...

Exemplary Claim: ...contain at least one N atom; and all pharmaceutically acceptable isomers, salts, hydrates, solvates and **prodrug** derivatives thereof.

Non-exemplary Claims: ...contain at least one N atom; and all pharmaceutically acceptable isomers, salts, hydrates, solvates and **prodrug** derivatives thereof...

...3. The compound of claim 1, having an IC50 for **Factor Xa** of less than about 200 nM...

?

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? ds

Set     Items   Description
S1      22392   PRODRUG??
S2      73467   THROMBIN
S3        140   S1 AND S2
S4          9   S3 AND PY=1998
S5          7   RD (unique items)
S6          1   S5 AND PY<=1997
S7      15536   FACTOR(W)VA OR FACTOR(W)VIIA OR FACTOR(W)IXA OR FACTOR(W)XA
              OR FACTOR XIIA
S8         84   S1 AND S7
S9          8   S8 AND PY<=1997
S10         7   RD (unique items)
S11    117513   TPA OR CVF OR C3B OR PROTEIN(W)C OR C(W)1S(W)INHIBITOR OR -
              ANTITRYPSIN OR HIRUDIN OR AT(W)II OR TFP(W)I OR PAI(W)1 OR PA-
              I(W)2 OR PAI(W)3 OR ONCOSTATIN(W)M
? s s1 and s11
           22392  S1
          117513  S11
     S12      57  S1 AND S11
? s s12 and py<=1997
Processing
              57  S12
        31493495  PY<=1997
     S13      28  S12 AND PY<=1997
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S14      26  RD (unique items)
? s s14 and py=1996
              26  S14
         2019184  PY=1996
     S15       6  S14 AND PY=1996
? s s14 and py<=1996
Processing
              26  S14
        29426269  PY<=1996
     S16      15  S14 AND PY<=1996
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S17      15  RD (unique items)
? t s17/3,k,ab/1-15

 17/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08778879   96129245   PMID: 8548555
  Assessment of bystander effect potency produced by intratumoral
implantation of HSVtk-expressing cells using surrogate marker secretion to
monitor tumor growth kinetics.
  Marini F C; Nelson J A; Lapeyre J N
  Department of Experimental Pathology, University of Texas MD Anderson
Cancer Center, Houston 77054, USA.
  Gene therapy (ENGLAND)   Nov 1995,  2  (9)  p655-9,  ISSN 0969-7128
Journal Code: 9421525
  Contract/Grant No.: CA 61948; CA; NCI; CA-16672; CA; NCI
  Document type: Journal Article
```

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

A molecular marker, human alpha-1-**antitrypsin** (hAAT) was transduced into tumor cells and its secretion was found to correlate with tumor growth or regression, allowing for an accurate and continuous measurement of tumor growth kinetics. Using this system, we investigated the therapeutic potential produced purely from the bystander effect of HSVtk+ CT26 cells to eradicate established CT26 colon carcinomas in mice by direct intratumoral implantation and subsequent ganciclovir administration. With lower ratios (0.1% and 1% of initial tumor burden), tumor growth kinetics went into a static (remission) phase of approximately 2 weeks duration before relapse and resumption of progressive tumor growth. When the number of CT26tk+ modified cells injected into the tumor equaled 10% to 100% of the initial tumor cell number the bystander effect was sufficient to completely eradicate established tumors indicating that a potent bystander killing effect is produced in this system, and that a cellular therapy based on this approach may have applications.

Nov **1995**,

A molecular marker, human alpha-1-**antitrypsin** (hAAT) was transduced into tumor cells and its secretion was found to correlate with tumor...

...Descriptors: Gene Therapy--methods--MT; *Thymidine Kinase --biosynthesis--BI; *Tumor Markers, Biological--secretion--SE; *alpha 1-**Antitrypsin**--secretion--SE...; Ganciclovir--administration and dosage --AD; Gene Transfer Techniques; Mice; Mice, Inbred BALB C; Neoplasm Transplantation; **Prodrugs**--administration and dosage--AD; Simplexvirus--enzymology--EN; Thymidine Kinase--genetics--GE; Tumor Markers, Biological--genetics--GE; alpha 1-**Antitrypsin**--genetics--GE

Chemical Name: Antineoplastic Agents; **Prodrugs**; Tumor Markers, Biological; alpha 1-**Antitrypsin**; Ganciclovir; Thymidine Kinase

17/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08609031 95369143 PMID: 7641602

Novel antithrombotic drugs in development.

Verstraete M; Zoldhelyi P

Center for Molecular and Vascular Biology, University of Leuven, Belgium.

Drugs (NEW ZEALAND) Jun **1995**, 49 (6) p856-84, ISSN 0012-6667
Journal Code: 7600076

Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Platelet activation plays a critical role in thromboembolic disorders, and aspirin remains a keystone in preventive strategies. This remarkable efficacy is rather unexpected, as aspirin selectively inhibits platelet aggregation mediated through activation of the arachidonic-thromboxane pathway, but not platelet aggregation induced by adenosine diphosphate (ADP), collagen and low levels of thrombin. This apparent paradox has stimulated investigations on the effect of aspirin on eicosanoid-independent effects of aspirin on cellular signalling. It has also fostered the search for antiplatelet drugs inhibiting platelet aggregation at other levels than the acetylation of platelet cyclo-oxygenase, such as thromboxane synthase inhibitors and thromboxane receptor antagonists. The final step of all platelet agonists is the functional expression of glycoprotein (GP) IIb/IIIa on the platelet surface, which ligates fibrinogen to link platelets together as part of the aggregation process. Agents that interact between GPIIb/IIIa and fibrinogen have been developed, which block GPIIb/IIIa, such as monoclonal antibodies to GPIIb/IIIa, and natural and synthetic peptides (disintegrins) containing

the Arg-Gly-Asp (RGD) recognition sequence in fibrinogen and other adhesion macromolecules. Also, some non-peptide RGD mimetics have been developed which are orally active **prodrugs**. Stable analogues of prostacyclin, some of which are orally active, are also available. Thrombin has a pivotal role in both platelet activation and fibrin generation. In addition to natural and recombinant human antithrombin III, direct antithrombin III-independent thrombin inhibitors have been developed as recombinant **hirudin** , hirulog, argatroban, boroarginine derivatives and single stranded DNA oligonucleotides (aptanes). Direct thrombin inhibitors do not affect thrombin generation and may leave some 'escaping' thrombin molecules unaffected. Inhibition of factor Xa can prevent thrombin generation and disrupt the thrombin feedback loop that amplifies thrombin production.

Jun **1995**,
...adhesion macromolecules. Also, some non-peptide RGD mimetics have been developed which are orally active **prodrugs** . Stable analogues of prostacyclin, some of which are orally active, are also available. Thrombin has...

... recombinant human antithrombin III, direct antithrombin III-independent thrombin inhibitors have been developed as recombinant **hirudin**, hirulog, argatroban, boroarginine derivatives and single stranded DNA oligonucleotides (aptanes). Direct thrombin inhibitors do not...
...; metabolism--ME; Molecular Sequence Data; Platelet Aggregation--drug effects--DE; Platelet Aggregation Inhibitors--pharmacology--PD; **Protein C**--metabolism--ME; Thrombomodulin; Thrombosis --drug therapy--DT; Thrombosis--prevention and control--PC; Ticlopidine--analogs and...
Chemical Name: Fibrinolytic Agents; Glycoproteins; Platelet Aggregation Inhibitors; **Protein C**; Thrombomodulin; Ticlopidine; clopidogrel

17/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07419333 92352501 PMID: 1642667
Effects of the new angiotensin-I-converting enzyme inhibitor imidapril on the responses of isolated vascular preparations to various agonists.
Kubo M; Fujitsuka T; Ishida R
Pharmacological Research Laboratory, Tanabe Seiyaku Co., Ltd., Osaka, Japan.
Arzneimittel-Forschung (GERMANY) Apr **1992**, 42 (4) p446-51,
ISSN 0004-4172 Journal Code: 0372660
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Effects of imidapril hydrochloride ((-)-(4S)-3-[(2S)-2-[[(1S)-1-ethoxycarbonyl-3-phenylpropyl]amino]propionyl]-1-methyl-2- oxoimidazolidine -4-carboxylic acid hydrochloride, imidapril, TA-6366, CAS 89396-94-1), a new **prodrug** type angiotensin converting enzyme (ACE) inhibitor, and 6366 A (CAS 89371-44-8), an active metabolite of imidapril, on isolated vascular preparations were studied. 6366 A inhibited angiotensin I (AT-I)-induced contraction of the rabbit thoracic aorta at 3 x 10(-10) mol/l or more and augmented bradykinin (BK)-induced relaxation of the dog renal artery precontracted with prostaglandin F2 alpha PGF2 alpha at 10(-9) mol/l or more, whereas imidapril at 10(-7) mol/l did not affect these responses. However, 6366 A, like imidapril, had no effect on angiotensin II (**AT-II**), norepinephrine, serotonin-, KCl- and PGF2 alpha-induced contractions. The inhibitory effect of 6366 A on AT-I-induced contraction was attenuated by denudation of the endothelium, but it was still maintained even after washing out the aorta that had been previously exposed to the medium containing 6366 A. This suggests that 6366 A

persistently inhibits the angiotensin I converting enzyme located preferentially in the endothelium. Therefore, the antihypertensive action of imidapril is mainly attributable to the vasodilation through the inhibitory effects of 6366 A on **AT-II** synthesis and BK degradation in the vasculature.

Apr **1992**,
... 2- oxoimidazolidine-4-carboxylic acid hydrochloride, imidapril, TA-6366, CAS 89396-94-1), a new **prodrug** type angiotensin converting enzyme (ACE) inhibitor, and 6366 A (CAS 89371-44-8), an active...

... not affect these responses. However, 6366 A, like imidapril, had no effect on angiotensin II (**AT-II**), norepinephrine, serotonin-, KCl- and PGF2 alpha-induced contractions. The inhibitory effect of 6366 A on...
... imidapril is mainly attributable to the vasodilation through the inhibitory effects of 6366 A on **AT-II** synthesis and BK degradation in the vasculature.

17/3,K,AB/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05441294 Genuine Article#: VZ167 Number of References: 226
Title: COAGULATION FOR BLOOD BANKERS (Abstract Available)
Author(s): STUBBS JR
Corporate Source: UNIV S ALABAMA,MED CTR,HEMOSTASIS LAB,2451 FILINGIM ST/MOBILE//AL/36617
Journal: CLINICS IN LABORATORY MEDICINE, **1996**, V16, N4 (DEC), P837&
ISSN: 0272-2712
Language: ENGLISH Document Type: REVIEW
Abstract: This article reviews the current understanding of coagulation and its role in overall hemostasis. Also provided is an overview of current screening and specialized tests of coagulation.

, **1996**
...Identifiers--ACTIVATED **PROTEIN-C**; PARTIAL THROMBOPLASTIN TIME; EXTRINSIC PATHWAY INHIBITOR; MOLECULAR-WEIGHT KININOGEN; FACTOR-VIII INHIBITORS; TISSUE FACTOR PATHWAY...
Research Fronts: 95-0625 014 (ACTIVATED **PROTEIN-C** RESISTANCE; VENOUS THROMBOSIS; FACTOR-V LEIDEN)
95-3491 004 (TISSUE FACTOR PATHWAY INHIBITOR; INTEGRIN REGULATION...

...RISK; CORONARY-ARTERY DISEASE; PATHOPHYSIOLOGY OF FIBRINOLYSIS)
95-5540 001 (PLASMA THROMBOMODULIN; DISSEMINATED INTRAVASCULAR COAGULATION; **PROTEIN-C** PATHWAY)
95-6130 001 (FACTOR-IX GENE; MUTATIONS CAUSING HEMOPHILIA-B; ACTIVATED **PROTEIN-C**; PROMOTER REGION)
95-7659 001 (DIAGNOSIS OF HEPATOCELLULAR-CARCINOMA; VITAMIN-K **PRODRUGS**; TISSUE FACTOR PATHWAY INHIBITOR)

17/3,K,AB/5 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

21/21

05179871 Genuine Article#: VF374 Number of References: 73
Title: DELIVERY OF PLASMID DNA TO ENDOTHELIAL-CELLS (Abstract Available)
Author(s): TAKAKURA Y; HASHIDA M
Corporate Source: KYOTO UNIV,DEPT DRUG DELIVERY RES/KYOTO//JAPAN/
Journal: EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, **1996**, V42, N4 (AUG), P269-276
ISSN: 0939-6411

Language: ENGLISH Document Type: ARTICLE
Abstract: The vascular endothelial cells are a particularly attractive target in the field of somatic gene therapy in which recombinant DNA is used as a macromolecular medicine. Because of their location, endothelial cells can be a reservoir that provides transgene products either systemically or locally and can be a reactant that inactivates a toxic substance or activates an inert **prodrug**. In this article, the current status of gene delivery to vascular endothelial cells will be reviewed. Methodologies and basic strategies for gene transfer to endothelial cells will be described and discussed from pharmaceutical and biopharmaceutical viewpoints. The feasibility of gene delivery systems in which plasmid DNA is combined with appropriate carrier systems to endothelial cells will be also discussed.
, **1996**
...Abstract: locally and can be a reactant that inactivates a toxic substance or activates an inert **prodrug**. In this article, the current status of gene delivery to vascular endothelial cells will be ...
...Identifiers--RECOMBINANT GENE-EXPRESSION; ADENOVIRUS-MEDIATED TRANSFER; ARTERIAL-WALL; DISPOSITION CHARACTERISTICS; ANTISENSE OLIGONUCLEOTIDES; HUMAN ALPHA-1-**ANTITRYPSIN**; INTIMAL HYPERPLASIA; LIPOSOME COMPLEXES; CATIONIC LIPOSOMES; RETROVIRAL VECTORS

17/3,K,AB/6 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05130827 Genuine Article#: VC063 Number of References: 17
Title: TEXTURE PROFILE ANALYSIS OF BIOADHESIVE POLYMERIC SEMISOLIDS - MECHANICAL CHARACTERIZATION AND INVESTIGATION OF INTERACTIONS BETWEEN FORMULATION COMPONENTS (Abstract Available)
Author(s): JONES DS; WOOLFSON AD; DJOKIC J
Corporate Source: QUEENS UNIV BELFAST,SCH PHARM,PHARMACEUT DEVICES GRP,CTR MED BIOL,97 LISBURN RD/BELFAST BT9 7BL/ANTRIM/NORTH IRELAND/
Journal: JOURNAL OF APPLIED POLYMER SCIENCE, **1996**, V61, N12 (SEP 19)
, P2229-2234
ISSN: 0021-8995
Language: ENGLISH Document Type: ARTICLE
Abstract: This study reports the use of texture profile analysis (**TPA**) to mechanically characterize polymeric, pharmaceutical semisolids containing at least one bioadhesive polymer and to determine interactions between formulation components. The hardness, adhesiveness, force per unit time required for compression (compressibility), and elasticity of polymeric, pharmaceutical semisolids containing polycarbophil (1 or 5% w/w), polyvinylpyrrolidone (3 or 5% w/w), and hydroxyethylcellulose (3, 5, or 10% w/w) in phosphate buffer (pH 6.8) were determined using a texture analyzer in the **TPA** mode (compression depth 15 mm, compression rate 8 mm s(-1) 15 s delay period). Increasing concentrations of polycarbophil, poly vinylpyrrolidone, and hydroxyethylcellulose significantly increased product hardness, adhesiveness, and compressibility but decreased product elasticity. Statistically, interactions between polymeric formulation components were observed within the experimental design and were probably due to relative differences in the physical states of polyvinylpyrrolidone and polycarbophil in the formulations, i.e., dispersed/dissolved and unswollen/swollen, respectively. Increased product hardness and compressibility were possibly due to the effects of hydroxyethylcellulose, polyvinylpyrrolidone, and polycarbophil on the viscosity of the formulations. Increased adhesiveness was related to the concentration and, more importantly, to the physical state of polycarbophil. Decreased product elasticity was due to the increased semisolid nature of the product. **TPA** is a rapid, straightforward analytical technique that may be applied to the

mechanical characterization of polymeric, pharmaceutical semisolids. It provides a convenient means to rapidly identify physicochemical interactions between formulation components. (C) 1996 John Wiley & Sons, Inc.

, **1996**
Abstract: This study reports the use of texture profile analysis (**TPA**) to mechanically characterize polymeric, pharmaceutical semisolids containing at least one bioadhesive polymer and to determine...

...w) in phosphate buffer (pH 6.8) were determined using a texture analyzer in the **TPA** mode (compression depth 15 mm, compression rate 8 mm s(-1) 15 s delay period...

...of polycarbophil. Decreased product elasticity was due to the increased semisolid nature of the product. **TPA** is a rapid, straightforward analytical technique that may be applied to the mechanical characterization of...
...Research Fronts: BIOADHESIVE POLYMER BUCCAL PATCHES; PROTEASE INHIBITORS IN RATS; PULMONARY DRUG-DELIVERY; ENZYMATIC DEGRADATION OF ACYCLOVIR **PRODRUGS**)

17/3,K,AB/7 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05075370 Genuine Article#: TN801 Number of References: 54
Title: SELECTIVE-INHIBITION OF THE REVERSE TRANSCRIPTION OF DUCK HEPATITIS-B VIRUS BY BINDING OF 2,3-DIDEOXYGUANOSINE 5-TRIPHOSPHATE TO THE VIRAL POLYMERASE (Abstract Available)
Author(s): HOWE AYM; ROBINS MJ; WILSON JS; TYRRELL DLJ
Corporate Source: UNIV ALBERTA,DEPT MED MICROBIOL & IMMUNOL/EDMONTON/AB T6G 2H7/CANADA/; UNIV ALBERTA,DEPT MED MICROBIOL & IMMUNOL/EDMONTON/AB T6G 2H7/CANADA/; UNIV ALBERTA,GLAXO HERITAGE RES INST/EDMONTON/AB T6G 2H7/CANADA/; BRIGHAM YOUNG UNIV,DEPT CHEM & BIOCHEM/PROVO//UT/00000
Journal: HEPATOLOGY, **1996**, V23, N1 (JAN), P87-96
ISSN: 0270-9139
Language: ENGLISH Document Type: ARTICLE
Abstract: Hepatitis B virus (HBV) replication is mediated by the viral polymerase that possesses three functional domains: primer, DNA polymerase/reverse transcriptase, and RNase H. Using the Pekin duck as an animal model, we demonstrate a novel mechanism of inhibition of duck hepatitis B virus (DHBV) by 2,6-diaminopurine 2',3'-dideoxyriboside (ddDAPR), a **prodrug** of 2',3'-dideoxyguanosine (ddG). A selective and irreversible inhibition of DHBV DNA replication is found in ducklings treated with high doses of ddDAPR (20 to 50 mg/kg), but not with similar doses of 2',3'-dideoxycytidine (ddC). The inhibition mediated by ddDAPR occurs at a very early stage of the reverse transcription, Despite the inhibition of DHBV DNA replication by ddDAPR, the DNA polymerase and reverse transcriptase activities of the polymerase are found to remain active when tested on exogenous templates in activity gels, We have demonstrated direct binding of [alpha-P-32]ddGTP to the DHBV polymerase expressed in an in vitro transcription and translation system. These results suggest that the binding of ddGTP to the polymerase blocks the initial DNA replication.

, **1996**
...Abstract: of duck hepatitis B virus (DHBV) by 2,6-diaminopurine 2',3'-dideoxyriboside (ddDAPR), a **prodrug** of 2',3'-dideoxyguanosine (ddG). A selective and irreversible inhibition of DHBV DNA replication is...
...Research Fronts: RESISTANCE MUTATIONS; ANTIRETROVIRAL THERAPY)
94-2087 001 (HEPATITIS-B VIRUS; Z-NUMBER-2 ALPHA(1)-**ANTITRYPSIN**

TRANSGENIC MICE; RAF-DEPENDENT ACTIVATION OF C-JUN TRANSCRIPTIONAL ACTIVITY)

17/3,K,AB/8 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04908551 Genuine Article#: UQ933 Number of References: 110
Title: HEPATOCELLULAR INJURY IN HEPATITIS-B AND HEPATITIS-C VIRUS-INFECTIONS (Abstract Available)
Author(s): FEITELSON MA
Corporate Source: THOMAS JEFFERSON UNIV,DEPT PATHOL ANAT & CELL BIOL,219 JEFFERSON ALUMNI HALL,1020 LOCUST ST/PHILADELPHIA//PA/19107
Journal: CLINICS IN LABORATORY MEDICINE, **1996**, V16, N2 (JUN), P307&
ISSN: 0272-2712
Language: ENGLISH Document Type: REVIEW
Abstract: Most of the liver cell injury in hepatitis B and C infections is likely to be immune-mediated. Variation in the pathogenesis of these infections likely is contributed by a variety of host and virus factors. Host factors include the human leukocyte antigen (HLA) haplotype as well as the ability of the host to both recognize antigen on virus-infected cells and to receive the appropriate costimulatory signals in a timely fashion during infection. Virus factors include the genetic variation, direct cytopathic effects, and the alteration of infected hepatocytes to cytotoxic cytokines. The lack of suitable tissue culture systems and animal models limits the ability to understand the pathogenesis fully but provides challenges for their future development so that the basis for liver cell damage can be elucidated and approaches for therapeutic intervention can be achieved.

, **1996**
...Research Fronts: ASPECTS OF HCV INFECTION)
94-2087 001 (HEPATITIS-B VIRUS; Z-NUMBER-2 ALPHA(1)-**ANTITRYPSIN** TRANSGENIC MICE; RAF-DEPENDENT ACTIVATION OF C-JUN TRANSCRIPTIONAL ACTIVITY)
94-3718 001 (HEPATITIS-B...

...7 PATIENTS)
94-8278 001 (PRIMARY RAT HEPATOCYTES; GENETIC TOXICOLOGY; RODENT LIVER; UNSCHEDULED DNA-SYNTHESIS; **PRODRUGS** OF AN ANTIVIRAL AGENT; COLLAGEN GEL IMMOBILIZATION)

17/3,K,AB/9 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04729630 Genuine Article#: UD418 Number of References: 95
Title: PULMONARY COMPLICATIONS OF LIVER-TRANSPLANTATION (Abstract Available)
Author(s): OBRIEN JD; ETTINGER NA
Corporate Source: WASHINGTON UNIV,SCH MED,DIV PULM & CRIT CARE MED,BOX 8052,660 S EUCLID ST/ST LOUIS//MO/63110; BARNES HOSP,DEPT INTERNAL MED/ST LOUIS//MO/63110; ST LUKES HOSP/ST LOUIS//MO/00000
Journal: CLINICS IN CHEST MEDICINE, **1996**, V17, N1 (MAR), P99&
ISSN: 0272-5231
Language: ENGLISH Document Type: ARTICLE
Abstract: With increasing numbers of patients undergoing liver transplantation for end-stage liver disease and continued improvements in survival rates, attention to the respiratory status of these patients has achieved even greater importance. This article discusses the preoperative pulmonary assessment for conditions that may prohibit liver transplantation or affect outcomes, in addition to discussing the

possible intraoperative and early infectious versus noninfectious postoperative complications. Also included is a discussion of the most common pulmonary complications that may be encountered late after liver transplantation.

, **1996**

...Identifiers--RESPIRATORY-DISTRESS SYNDROME; PRIMARY BILIARY-CIRRHOSIS; HEPATOPULMONARY SYNDROME; ALPHA-1-**ANTITRYPSIN** DEFICIENCY; CYTOMEGALOVIRUS-INFECTION; PORTAL-HYPERTENSION; CONTROLLED TRIAL; RECIPIENTS; DISEASE; DYSFUNCTION

Research Fronts: 94-1286 003 (CYTOMEGALOVIRUS DISEASE; CMV INFECTIONS IN ALLOGENEIC BONE-MARROW TRANSPLANT RECIPIENTS; ACYCLOVIR **PRODRUG** VALACICLOVIR; GANCICLOVIR PROPHYLAXIS)

94-2465 001 (INTRAPULMONARY SHUNTING CAUSING HYPOXEMIA; CHRONIC LIVER-DISEASE; HYPERDYNAMIC CIRCULATION...

17/3,K,AB/10 (Item 7 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00896998 Genuine Article#: FD869 Number of References: 24

Title: INHIBITION OF PYROGLUTAMYL PEPTIDASE-II SYNTHESIS BY PHORBOL ESTER IN THE Y-79 RETINOBLASTOMA CELL (Abstract Available)

Author(s): SUEN CS; WILK S

Corporate Source: CUNY MT SINAI SCH MED,DEPT PHARMACOL,1 GUSTAVE L LEVY PL/NEW YORK//NY/10029; CUNY MT SINAI SCH MED,DEPT PHARMACOL,1 GUSTAVE L LEVY PL/NEW YORK//NY/10029

Journal: ENDOCRINOLOGY, **1991**, V128, N4, P2169-2174

Language: ENGLISH Document Type: ARTICLE

Abstract: Pyroglutamyl peptidase II (EC 3.4.19.-), a highly specific membrane-bound TRH-degrading enzyme, is inactivated in Y-79 human retinoblastoma cells by exposure to 12-O-tetradecanoyl phorbol-13-acetate (**TPA**) in a biphasic manner. We have previously demonstrated a rapid decrease in pyroglutamyl peptidase II activity to 10% of the control level within 15 min, which returns to 70% of the control level by 1 h. This decrease results from enzyme phosphorylation by **TPA**-activated protein kinase-C. We now report a second phase of inactivation after longer exposure of cells to **TPA**. After 1 h, enzymatic activity slowly and progressively declined. By 7 h, only 15% of control activity remained. Cotreatment of cells with H-7, a protein kinase-C inhibitor, prevented this second phase of inactivation. Immunoblot experiments demonstrated a reduction in the amount of pyroglutamyl peptidase II in Y-79 membranes after long term exposure to **TPA**. Y-79 cells were labeled with [S-35]methionine, and pyroglutamyl peptidase II was immunoprecipitated. A decreased incorporations of [S-35]methionine paralleled the decrease in enzyme activity. These studies demonstrate that the second phase of inactivation after exposure to **TPA** is due to an inhibition of enzyme synthesis.

, **1991**

...Abstract: in Y-79 human retinoblastoma cells by exposure to 12-O-tetradecanoyl phorbol-13-acetate (**TPA**) in a biphasic manner. We have previously demonstrated a rapid decrease in pyroglutamyl peptidase II...

...70% of the control level by 1 h. This decrease results from enzyme phosphorylation by **TPA**-activated protein kinase-C. We now report a second phase of inactivation after longer exposure of cells to **TPA**. After 1 h, enzymatic activity slowly and progressively declined. By 7 h, only 15% of...

...the amount of pyroglutamyl peptidase II in Y-79 membranes after long term exposure to **TPA**. Y-79 cells were labeled with

[S-35]methionine, and pyroglutamyl peptidase II was immunoprecipitated ...

...in enzyme activity. These studies demonstrate that the second phase of inactivation after exposure to **TPA** is due to an inhibition of enzyme synthesis.
...Research Fronts: OF RAT EMBRYO; THYROTROPIN-RELEASING-HORMONE (TRH); PRECURSOR PEPTIDES; DOPAMINERGIC REGULATION)
89-1688 001 (MACROMOLECULAR **PRODRUGS**; TARGETED DRUG DELIVERY SYSTEMS; PYROGLUTAMYL AMINOPEPTIDASE; HYDROLYSIS KINETICS; THYROTROPIN RELEASING HORMONE DEGRADING ENZYMES)

17/3,K,AB/11 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00773147 Genuine Article#: EV522 Number of References: 15
Title: INTERFERENCE OF XANTHATE COMPOUNDS WITH PHORBOL ESTER **TPA** -INDUCED CHANGES OF PHOSPHOLIPID-METABOLISM - INHIBITION OF PROSTAGLANDIN PRODUCTION
Author(s): KASZKIN M; FURSTENBERGER G; KINZEL V
Corporate Source: GERMAN CANC RES CTR,INST BIOCHEM,NEUENHEIMER FELD 280/D-6900 HEIDELBERG 1//FED REP GER/; GERMAN CANC RES CTR,INST BIOCHEM,NEUENHEIMER FELD 280/D-6900 HEIDELBERG 1//FED REP GER/; GERMAN CANC RES CTR,INST EXPTL PATHOL/D-6900 HEIDELBERG 1//FED REP GER/
Journal: BIOCHEMICAL PHARMACOLOGY, **1991**, V41, N2, P315-318
Language: ENGLISH Document Type: NOTE

Title: INTERFERENCE OF XANTHATE COMPOUNDS WITH PHORBOL ESTER **TPA** -INDUCED CHANGES OF PHOSPHOLIPID-METABOLISM - INHIBITION OF PROSTAGLANDIN PRODUCTION
, **1991**
...Research Fronts: TRANSDUCTION; RAS ONCOGENE)
89-3010 001 (ANTIVIRAL AGENTS; ANTITUMORAL ACTIVITY OF A XANTHATE COMPOUND; ORAL **PRODRUG** (BRL-42810 FAMCICLOVIR))

17/3,K,AB/12 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00678235 Genuine Article#: EM456 Number of References: 47
Title: INDEPENDENT ASSOCIATION OF SERUM AMYLOID-P COMPONENT, PROTEIN-S, AND COMPLEMENT C4B WITH COMPLEMENT C4B-BINDING PROTEIN AND SUBSEQUENT ASSOCIATION OF THE COMPLEX WITH MEMBRANES
Author(s): SCHWALBE RA; DAHLBACK B; NELSESTUEN GL
Corporate Source: UNIV MINNESOTA,DEPT BIOCHEM/ST PAUL//MN/55108; MALMO GEN HOSP,DEPT CLIN CHEM/S-21401 MALMO//SWEDEN/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1990**, V265, N35, P 21749-21757
Language: ENGLISH Document Type: ARTICLE

, **1990**
Research Fronts: 88-0087 001 (**PROTEIN-C** DEFICIENCY; DEEP VENOUS THROMBOSIS; LONG-TERM ORAL ANTICOAGULANT-THERAPY)
88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG**-BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)
88-1014 001 (B-CELL STIMULATORY FACTORS...

17/3,K,AB/13 (Item 10 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00527027 Genuine Article#: DZ950 Number of References: 46
Title: ASSEMBLY OF PROTEIN-S AND C4B-BINDING PROTEIN ON MEMBRANES
Author(s): SCHWALBE R; DAHLBACK B; HILLARP A; NELSESTUEN G
Corporate Source: UNIV MINNESOTA,DEPT BIOCHEM/ST PAUL//MN/55108; UNIV LUND,MALMO GEN HOSP,DEPT CLIN CHEM/S-21401MALMO//SWEDEN/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1990**, V265, N27, P 16074-16081
Language: ENGLISH Document Type: ARTICLE

, **1990**
Research Fronts: 88-0087 002 (**PROTEIN-C** DEFICIENCY; DEEP VENOUS THROMBOSIS; LONG-TERM ORAL ANTICOAGULANT-THERAPY)
88-0643 002 (LIPOPHILIC MITOMYCIN-C **PRODRUG**-BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)

17/3,K,AB/14 (Item 11 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00351459 Genuine Article#: DK851 Number of References: 67
Title: MULTIPLE ACTIVE FORMS OF THROMBIN .4. RELATIVE ACTIVITIES OF MEIZOTHROMBINS
Author(s): DOYLE MF; MANN KG
Corporate Source: UNIV VERMONT,COLL MED,DEPT BIOCHEM,GIVEN BLDG,HLTH SCI COMPLEX/BURLINGTON//VT/05405; UNIV VERMONT,COLL MED,DEPT BIOCHEM,GIVEN BLDG,HLTH SCI COMPLEX/BURLINGTON//VT/05405
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1990**, V265, N18, P 10693-10701
Language: ENGLISH Document Type: ARTICLE

, **1990**
Research Fronts: 88-0087 002 (**PROTEIN-C** DEFICIENCY; DEEP VENOUS THROMBOSIS; LONG-TERM ORAL ANTICOAGULANT-THERAPY)
88-0643 001 (LIPOPHILIC MITOMYCIN-C **PRODRUG**-BEARING LIPOSOMES; FUSION OF LIPID VESICLES; DRUG DELIVERY)
88-1153 001 (RABBIT PLATELETS; IMMUNOCYTOCHEMICAL LOCALIZATION...

17/3,K,AB/15 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3327555 IFI Acc No: 0015882
Document Type: C
GENE THERAPY FOR SOLID TUMORS USING ADENOVIRAL VECTORS COMPRISING SUICIDE GENES AND CYTOKINE GENES; TOGETHER WITH **PRODRUG**
Inventors: Chen Shu-Hsia (US); Woo Savio L C (US)
Assignee: Baylor College of Medicine
Assignee Code: 06345
Publication (No,Date), Applic (No,Date):
US 6066624 20000523 US 96600942 19960215
Publication Kind: A
Calculated Expiration: 20170523
(Cited in 001 later patents)
Cont.-in-part Pub(No),Applic(No,Date): US 5631236 US 93112745 19930826
PCT Pub(No,Date),Applic(No,Date): WO 955835 **19950302** WO 94US9784 19940825
Section 371: 19960215
Section 102(e):19960215
Priority Applic(No,Date): US 96600942 19960215; US 93112745 19930826

Abstract: The present invention provides a novel method of treating localized solid tumors and papillomas in an individual, as well as metastatic carcinomas. The method comprises delivering a suicide gene, by way of a recombinant adenoviral vector or other DNA transport system, into the tumor, papilloma or wart of an individual. Subsequently, a **prodrug**, such as the drug gaciclovir(tm), is administered to the individual. Additionally, the present invention provides a method for treating solid tumors, papillomas, warts and metastatic carcinomas, said method comprising introducing both a suicide gene and one or more cytokine genes into the tumor, papilloma or wart of an individual, and subsequently administering a **prodrug** to the individual. The methods of the present invention may be used to treat several different types of cancers and papillomas, including colon carcinoma, prostate cancer, breast cancer, lung cancer, melanoma, hepatoma, brain lymphoma and head and neck cancer.

...TOGETHER WITH **PRODRUG**
...PCT Pub(No,Date),Applic(No,Date): **19950302**

Abstract: ...other DNA transport system, into the tumor, papilloma or wart of an individual. Subsequently, a **prodrug**, such as the drug gaciclovir(tm), is administered to the individual. Additionally, the present invention...
...cytokine genes into the tumor, papilloma or wart of an individual, and subsequently administering a **prodrug** to the individual. The methods of the present invention may be used to treat several...

Exemplary Claim: ...tumor expresses said suicide gene and said one or more cytokine genes; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene.
Non-exemplary Claims: ...suicide gene sequence to be expressed is thymidine kinase of herpes simplex virus and the **prodrug** is ganciclovir, acyclovir, FIAU or their derivatives...

...1, wherein the suicide gene sequence to be expressed is bacteria cytosine deaminase and the **prodrug** is 5-fluorocytosine or its derivatives...

...the suicide gene sequence to be expressed is varicella zoster virus thymidine kinase and the **prodrug** is 6-methoxypurine arabinoside or its derivatives...

...10. The method of claim 1, wherein said **prodrug** is selected from the group consisting of ganciclovir, acyclovir, 1-5-iodouracil FIAU, 5-fluorocytosine...linked to a promoter and wherein said tumor expresses the cytokine gene; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene...

...suicide gene sequence to be expressed is thymidine kinase of herpes simplex virus and the **prodrug** is ganciclovir, acyclovir, FIAU or their derivatives...

...14, wherein the suicide gene sequence to be expressed is bacterial cytosine deaminase and the **prodrug** is 5-fluorocytosine or its derivatives...

...the suicide gene sequence to be expressed is varicella zoster virus thymidine kinase and the **prodrug** is 6-methoxypurine arabinoside or its derivatives...

...selected from the group consisting of an albumin promoter, an alpha-fetoprotein promoter, an alpha -**antitrypsin** promoter and a

phosphoenol pyruvate carboxykinase promoter...36. The method of claim 14, wherein said **prodrug** is selected from the group consisting of ganciclovir, acyclovir, 1-5-iodouracil FIAU, 5-fluorocytosine...wherein said tumor expresses said suicide gene and said IL-2 gene; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene...

...linked to a promoter and wherein said tumor expresses the cytokine gene; and administering a **prodrug** in amounts sufficient to cause regression of said tumor when said **prodrug** is converted to a toxic compound by said suicide gene...

?

0276171 20090109

Generation of PSA-ACT-specific monoclonal antibodies and their application in a sandwich immunoassay.

Wang TJ; Linton HJ; Payne J; Rittenhouse HG; Chan DW; Partin AW; Wolfert RL; Kuus-Reichel K

Department of Research and Development, Hybritech Incorporated, San Diego, CA 92121, USA. tjwang@beckman.com

Hybridoma (UNITED STATES) Dec 1999, 18 (6) p535-41, ISSN 0272-457X Journal Code: GFS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The prostate-specific antigen (PSA) immunoassay is an important tool for the detection and monitoring of prostate cancer. PSA exists in **serum** mainly as **complexes** with serine **protease inhibitors** including alpha1 antichymotrypsin (ACT) and alpha2 macroglobulin (MG). PSA-MG **complex** is not detected by the existing PSA immunoassays since MG (720 kDa) sequesters PSA and masks the antibody binding sites. Existing immunoassays for quantitation of total **serum** PSA measure PSA-ACT (CPSA) and free PSA (FPSA), which comprise the major and minor components of total PSA, respectively. Monoclonal antibodies (MAb) specific for CPSA alone were generated using a unique immunogen prepared by blocking the major antigenic determinants on FPSA and ACT. The blocked immunogen greatly enhanced the frequency of hybridomas reactive against the CPSA complex. CPSA prototype immunoassays were established using anti-CPSA (PX1G359) or anti-ACT (AC1A212) MAb as tracer MAb and anti-PSA (PSA399) MAb as capture MAb. The complex-selective MAbs demonstrated minimal cross-reactivity with **Cathepsin**-G (CG) ACT (CG-ACT), ACT or FPSA. CG-ACT complex interfered with the accuracy of initial prototype assays specific for CPSA measurement and caused over-recovery (1 to 3 ng/mL, with 40 to 180 ng/mL range of CG-ACT in **serum**) of apparent CPSA values. Addition of 0.4% NP-40 and 0.1% 0.088 micron microparticles in clinical specimens eliminated this interference. Specimens from 39 prostate cancer (PCa) patients and 44 benign prostatic hyperplasia (BPH) patients were analyzed with the PX1G359 CPSA assay. In this study, the area under the curve (AUC) values for ROC analysis of total PSA (CPSA+FPSA), FPSA to total PSA ratio (f/t), and FPSA to CPSA ratio (f/c) were 0.357, 0.634, and 0.624, respectively. In a second study using AC1A212 CPSA assay, where specimens from 16 PCa patients and 48 BPH patients were tested, the AUC values for total PSA, f/t and f/c ratios were 0.62, 0.785, and 0.732, respectively. Using the CPSA assay with minimal interference our studies are consistent with previous CPSA data showing that the f/t PSA ratio remains superior to the f/c PSA ratio in differentiating PCa and BPH diseases. Complex PSA by itself or as ratio with free or total PSA does not provide any advantage over the established method of FPSA to total PSA ratio.

... is an important tool for the detection and monitoring of prostate cancer. PSA exists in **serum** mainly as **complexes** with serine

25/3,K,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04475248 84197067

Inhibition of the human tissue plasminogen activator in plasma from different species.

Haggroth L; Mattsson C; Friberg J

Thrombosis research (UNITED STATES) Mar 15 1984, 33 (6) p583-94, ISSN 0049-3848 Journal Code: VRN
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The rate and extent of complex formation between protease inhibitors present in plasma from different species and a human plasminogen activator purified to homogeneity from the supernatant of a human melanoma cell line was studied in vitro. The fibrinolytic activity of the one-chain plasminogen activator disappeared form human, cat and rabbit plasma with a half-life of 100 minutes. By means of antibodies directed against purified protease inhibitors the main inhibitor in human, cat, dog and rabbit plasma was identified as alpha 2-antiplasmin. Alpha 2-macroglobulin and C1-esterase-inhibitor functioned as inhibitors to a lesser extent. The main inhibitor in rat plasma was alpha 2-macroglobulin. The plasma half-life was 3 (rabbit and human) to 10 (dog and rat) times shorter for the two-chain form than for the one-chain form of the plasminogen activator molecule. It is also concluded that, with respect to plasma elimination of the fibrinolytic activity, among the common laboratory animals, the rabbit is the most suitable animal available for the study of fibrinolysis.

Inhibition of the human tissue plasminogen activator in plasma from different species.
The rate and extent of complex formation between protease inhibitors present in plasma from different species and a human plasminogen activator purified to homogeneity from the supernatant of a human melanoma cell line was studied in vitro. The fibrinolytic activity of the one-chain plasminogen activator disappeared form human, cat and rabbit plasma with a half-life of 100 minutes. By...

...times shorter for the two-chain form than for the one-chain form of the plasminogen activator molecule. It is also concluded that, with respect to plasma elimination of the fibrinolytic activity...

; alpha-Macroglobulins--Metabolism--ME; Antiplasmin--Metabolism--ME; Cats ; Complement 1 Inactivators--Blood--BL; Dogs; Fibrinolysis; Half-Life ; Plasminogen Activators--Blood--BL; Protease Inhibitors--Blood

25/3,K,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06298708 86199741

On the role of proteases, their inhibitors and the extracellular matrix in promoting neurite outgrowth.

Patterson PH

Journal de physiologie (FRANCE) 1985, 80 (4) p207-11, ISSN 0021-7948 Journal Code: JRB
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Using a novel method, a monoclonal antibody was produced which can directly block the activity of an extracellular matrix-associated neurite outgrowth promoting complex (Matthew and Patterson, 1983). Presumably binding at or near the active site, this antibody recognizes a determinant consisting of heparan sulfate and a larger molecule which is likely to be laminin (Matthew et al., in preparation). The antibody has been further used to localize this determinant in adult tissues in vivo. Extracellular binding is seen at sites known to promote axon regeneration in the peripheral nervous system and is not seen in the central nervous system (Matthew et al., in preparation). In investigating how neurons may modify their environment as they grow processes, we have recently found that sensory and sympathetic neurons spontaneously release a collagenase and a plasminogen activator from their distal processes and/or growth cones (Pittman, 1985). A 43 kD irreversible inhibitor of the plasminogen activator is secreted by cardiac myocytes and is found on the surfaces of cultured neurons (Pittman, 1984). This inhibitor is also released by nonneuronal cell cultures from peripheral, but not central, nerves (Pittman, unpublished). Of interest in relation to the proteoglycan neurite outgrowth promoting complex is the finding that the 43 kD inhibitor preparation binds heparin tightly and can displace laminin from its heparin binding site (Patterson and Pittman, unpublished). Thus it is possible that the protease/inhibitor system could affect outgrowth via interaction with the neurite outgrowth promoting complex in the extracellular matrix.

On the role of proteases, their inhibitors and the extracellular matrix in promoting neurite outgrowth.

... novel method, a monoclonal antibody was produced which can directly block the activity of an extracellular matrix-associated neurite outgrowth promoting complex (Matthew and Patterson, 1983). Presumably b

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SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2003/Feb W3
         (c) format only 2003 The Dialog Corp.
  File  55:Biosis Previews(R)  1993-2003/Feb W3
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*File  55: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
  File  34:SciSearch(R) Cited Ref Sci  1990-2003/Feb W3
         (c) 2003 Inst for Sci Info
*File  34: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
  File 434:SciSearch(R) Cited Ref Sci  1974-1989/Dec
         (c) 1998 Inst for Sci Info
  File 340:CLAIMS(R)/US Patent  1950-03/Feb 20
         (c) 2003 IFI/CLAIMS(R)
*File 340: The Claims U.S. Patent databases have been reloaded.
 HELP NEWS340 & HELP ALERTS340 for search, display & Alert info.

      Set  Items  Description
      ---  -----  -----------
? s prodrug??
      S1   22393  PRODRUG??
? s endogenous(5n) (peptide or polypeptide or protein)
          293771  ENDOGENOUS
          626844  PEPTIDE
          173562  POLYPEPTIDE
         3125396  PROTEIN
      S2   19047  ENDOGENOUS(5N) (PEPTIDE OR POLYPEPTIDE OR PROTEIN)
? s s1 and s2
           22393  S1
           19047  S2
      S3      15  S1 AND S2
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S4       9  RD (unique items)
? s s4 and py<=1997
Processing
Processing
               9  S4
        31493495  PY<=1997
      S5       5  S4 AND PY<=1997
? t s5/3,k,ab/1-5

 5/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2003 The Dialog Corp. All rts. reserv.

08088770   94217171   PMID: 8164250
  New  dual  inhibitors of neutral endopeptidase and angiotensin-converting
enzyme:  rational design, bioavailability, and pharmacological responses in
experimental hypertension.
  Fournie-Zaluski M C; Coric P; Turcaud S; Rousselet N; Gonzalez W; Barbe B
; Pham I; Jullian N; Michel J B; Roques B P
  Unite de Pharmacochimie Moleculaire et Structurale, U266 INSERM-URA D1500
CNRS, UFR des Sciences Pharmaceutiques et Biologiques, Paris, France.
  Journal  of  medicinal chemistry (UNITED STATES)   Apr 15 1994,  37
 (8)  p1070-83,  ISSN 0022-2623   Journal Code: 9716531
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
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Record type: Completed

In the treatment of cardiovascular diseases, it could be of therapeutic interest to associate the hypotensive effects resulting from the inhibition of angiotensin II formation, ensured by endothelial angiotensin-converting enzyme (ACE), with the diuretic and natriuretic responses due to the protection of the **endogenous** atrial natriuretic **peptide** (ANP) from inactivation by epithelial neutral endopeptidase (NEP). However, an investigation of this hypothesis requires an orally active compound able to jointly inhibit ACE and NEP. Dual inhibitors have therefore been designed by a rational approach, based on the characteristics of the active sites of both enzymes, which belong to the same family of zinc metallopeptidases, and on the structures of their most potent and selective inhibitors. As both NEP and ACE contain a large S'1-S'2 domain able to accommodate aromatic residues, the cyclic ACE inhibitor 3-(mercaptomethyl)-3,4,5,6-tetrahydro-2-oxo-1H-1-benzazocine-1-ace tic acid was selected as a template. Various aliphatic constraints were introduced on the benzyl moiety of the potent NEP inhibitor N-[2-(mercaptomethyl)-3-phenylpropanoyl]-L-tyrosine (IC50 NEP = 2 nM, IC50 ACE = 25 nM) to improve the fit between the computed most stable conformers of these molecules and the ACE template. New dual inhibitors, of general formula, N-[2(R,S)-(mercaptomethyl)-3(R,S)-phenylbutanoyl]-L-amino acid with IC50 values in the nanomolar range for both enzymes were generated by this approach. The separation of the four stereoisomers using chiral amines and the stereoselective synthesis of the 2-(mercaptomethyl)-3-phenylbutanoyl moiety showed that inhibitors with the 2S,3R configuration are the most potent on both NEP and ACE. The "in vivo" potency of various **prodrugs** of these inhibitors to inhibit ACE activity in lung and NEP activity in kidney was measured after oral administration in mice. From this pharmacokinetical study the most potent dual inhibitor RB 105 (N-[(2S,3R)-2-(mercaptomethyl)-3-phenylbutanoyl-L-alanine (compound 44c) (KI NEP 1.7 nM, KI ACE 4.5 nM) and its most efficient in vivo **prodrug** mixanpril, [N-[(2S,3R)-2-[(benzoylthio)methyl]-3-phenylbutanoyl]-L-alan ine (compound 18) (ED50 NEP approximately 1 mg/kg, ED50 ACE approximately 7 mg/kg) were selected. Competition experiments with a tritiated inhibitor of ACE or NEP bound to mouse lung and kidney membranes respectively showed that mixanpril has a long duration of action (> 8 h). As expected, after i.v. administration in the spontaneously hypertensive rat (SHR), RB 105 decreased blood pressure and increased diuresis and natriuresis.(ABSTRACT TRUNCATED AT 400 WORDS)

Apr 15 **1994**,

... converting enzyme (ACE), with the diuretic and natriuretic responses due to the protection of the **endogenous** atrial natriuretic **peptide** (ANP) from inactivation by epithelial neutral endopeptidase (NEP). However, an investigation of this hypothesis requires...

... are the most potent on both NEP and ACE. The "in vivo" potency of various **prodrugs** of these inhibitors to inhibit ACE activity in lung and NEP activity in kidney was...

...NEP 1.7 nM, KI ACE 4.5 nM) and its most efficient in vivo **prodrug** mixanpril, [N-[(2S,3R)-2-[(benzoylthio)methyl]-3-phenylbutanoyl]-L-alan ine (compound 18) (ED50...

...; Lung--enzymology--EN; Mice; Models, Molecular; Molecular Sequence Data; Molecular Structure; Natriuresis--drug effects--DE; **Prodrugs**; Rabbits; Rats; Rats, Inbred SHR; Recombinant Proteins; Stereoisomerism

Chemical Name: Angiotensin-Converting Enzyme Inhibitors; **Prodrugs**; Recombinant Proteins; N-(2-(mercaptomethyl)-3-phenylbutanoyl)-L-alanine; Alanine; Neprilysin

5/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08083287 94224885 PMID: 8171037

Dual inhibition of angiotensin-converting enzyme and neutral endopeptidase by the orally active inhibitor mixanpril: a potential therapeutic approach in hypertension.

Fournie-Zaluski M C; Gonzalez W; Turcaud S; Pham I; Roques B P; Michel J B

Departement de Pharmacochimie Moleculaire et Structurale, U266, Institut National de la Sante et de la Recherche Medicale, URA D 1500, Centre National de la Recherche Scientifique, Faculte de Pharmacie, Paris, France.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr 26 **1994**, 91 (9) p4072-6, ISSN 0027-8424 Journal Code: 7505876

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

In the treatment of cardiovascular disease, it could be of therapeutic interest to associate the hypotensive effects due to the inhibition of angiotensin II formation with the diuretic and natriuretic responses induced by the protection of the **endogenous** atrial natriuretic **peptide** (ANP). Investigation of this hypothesis requires an orally active compound able to simultaneously inhibit angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP), which is involved in renal ANP metabolism. Such compounds have been rationally designed by taking into account the structural characteristics of the active site of both peptidases. Among them, RB 105, N-[(2S,3R)-2-mercaptomethyl-1-oxo-3-phenylbutyl]-(S)-alanine, inhibited NEP and ACE with Ki values of 1.7 +/- 0.3 nM and 4.2 +/- 0.5 nM, respectively. Intravenous infusion of RB 105 in conscious spontaneously hypertensive rats prevented the pressor response to exogenous angiotensin I and potentiated the natriuretic response to ANP. Infusion of RB 105, at 2.5, 5, 10, 25, and 50 mg/kg per hr decreased blood pressure dose-dependently in conscious catheterized spontaneously hypertensive rats and increased diuresis and natriuresis. Infusion of RB 105 as a bolus of 25 mg/kg followed by 25 mg/kg per hr similarly decreased blood pressure and increased natriuresis in three different models of hypertension (renovascular, deoxycorticosterone acetate-salt, and spontaneously hypertensive rats). Mixanpril, a lipophilic **prodrug** of RB 105 (ED50 values when given orally to mice, 0.7 mg/kg for NEP; 7 mg/kg for ACE), elicited dose-dependent hypotensive effects of long duration in spontaneously hypertensive rats after oral administration [-37 mmHg for 50 mg/kg twice a day (1 mmHg = 133 Pa) and is therefore the first dual NEP/ACE inhibitor potentially useful for clinical investigations.

Apr 26 **1994**,

... angiotensin II formation with the diuretic and natriuretic responses induced by the protection of the **endogenous** atrial natriuretic **peptide** (ANP). Investigation of this hypothesis requires an orally active compound able to simultaneously inhibit angiotensin...

... different models of hypertension (renovascular, deoxycorticosterone acetate-salt, and spontaneously hypertensive rats). Mixanpril, a lipophilic **prodrug** of RB 105 (ED50 values when given orally to mice, 0.7 mg/kg for...

...Descriptors: derivatives--AA; *Angiotensin-Converting Enzyme Inhibitors; *Hypertension--drug therapy--DT; *Neprilysin--antagonists and inhibitors--AI; ***Prodrugs**--administration and dosage--AD

Chemical Name: Angiotensin-Converting Enzyme Inhibitors; **Prodrugs**; N-(2-(mercaptomethyl)-3-phenylbutanoyl)-L-alanine; mixanpril; Alanine; Neprilysin

5/3,K,AB/3 (Item 1 from file: 34)

05579598 Genuine Article#: WH748 Number of References: 34
Title: Ortho-substituted benzofused macrocyclic lactams as zinc metalloprotease inhibitors (ABSTRACT AVAILABLE)
Author(s): Ksander GM (REPRINT) ; deJesus R; Yuan A; Ghai RD; Trapani A; McMartin C; Bohacek R
Corporate Source: NOVARTIS PHARMACEUT CORP,RES DEPT, 556 MORRIS AVE/SUMMIT//NJ/07901 (REPRINT)
Journal: JOURNAL OF MEDICINAL CHEMISTRY, **1997**, V40, N4 (FEB 14), P 495-505
ISSN: 0022-2623 Publication date: 19970214
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036
Language: English Document Type: ARTICLE
Abstract: The design and preparation of ortho-substituted benzofused macrocyclic lactams are described. The benzofused macrocyclic lactams were designed as neutral endopeptidase 24,11 (NEP) inhibitors. Docking studies were carried out in a model of thermolysin (TLN) using the MACROMODEL and QXP modeling programs to select suitable ring sizes. These studies predicted that the 11-, 12-, and 13-membered ring macrocyclic lactams would be active in both enzymes TLN and NEP. Good predictability of experimental results, within this series, of binding to thermolysin and to a lesser extent to NEP was observed. A visual comparison, docked at the active site of TLN, is presented for thiorphan, a 10-membered ring;macrocycle and an 11-membered ring benzofused macrocyclic lactam. Potent inhibition of both NEP and thermolysin was obtained. The 11-membered ring macrocycle 25a is the most potent inhibitor from this series of compounds (TLN IC50 = 68 nM; NEP IC50 = 0.9 nM). The effects of **prodrug** 44b administered at 10 mg/kg po on plasma atrial natriuretic peptide (ANP) levels in conscious rats was greater than 200% over a 4 h period.

, **1997**
...Abstract: series of compounds (TLN IC50 = 68 nM; NEP IC50 = 0.9 nM). The effects of **prodrug** 44b administered at 10 mg/kg po on plasma atrial natriuretic peptide (ANP) levels in...
...Research Fronts: GELATINOLYTIC ACTIVITY; TISSUE INHIBITOR)
95-1556 001 (NEUTRAL ENDOPEPTIDASE INHIBITOR; CCKB RECEPTOR ANTAGONIST; ROLE OF **ENDOGENOUS** CHOLECYSTOKININ)
95-1744 001 (NATRIURETIC **PEPTIDE** RECEPTORS; RAT VASCULAR ENDOTHELIAL-CELL MIGRATION; CALCIUM BLOCKER DOWN-REGULATES GENE-EXPRESSION)
95-2430 001...

5/3,K,AB/4 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03271989 Genuine Article#: NR957 Number of References: 49
Title: EFFECTS OF OPIOIDS AND NON-OPIOIDS ON C-FOS-LIKE IMMUNOREACTIVITY INDUCED IN RAT LUMBAR SPINAL-CORD NEURONS BY NOXIOUS HEAT STIMULATION (Abstract Available)
Author(s): ABBADIE C; HONORE P; FOURNIEZALUSKI MC; ROQUES BP; BESSON JM
Corporate Source: INSERM,U161,PHYSIOPHARMACOL SYST NEWVEUX LAB,2 RUE ALESIA/F-75014 PARIS//FRANCE/; EPHE/F-75014 PARIS//FRANCE/; PHARMACOCHIM MOLEC & STRUCT LAB,CNRS,URA D 1500,INSERM,U266/F-75270 PARIS 06//FRANCE/
Journal: EUROPEAN JOURNAL OF PHARMACOLOGY, **1994**, V258, N3 (JUN 13), P 215-227
ISSN: 0014-2999
Language: ENGLISH Document Type: ARTICLE
Abstract: This study evaluated Fos-like immunoreactivity in rat lumbar spinal cord neurons following peripheral noxious heat stimulation and the modifications induced by pharmacological agents. Under urethane anaesthesia, the hindpaw was stimulated by dipping it in a regulated

temperature bath at various temperatures (44-65 degrees C) and for various durations (5 s to 2 min). There was no Fos-like immunoreactivity in lumbar spinal cord neurons when the paw was stimulated at 44 degrees C for 15 s. From 46 to 52 degrees C, the number of Fos-like immunoreactivity neurons increased with increasing stimulation temperature, but was decreased at 65 degrees C as compared to 52 degrees C. At 52 degrees C, the number of Fos-like immunoreactivity neurons increased with the duration of stimulation. Fos-like immunoreactive neurons in the L4 segment were almost exclusively located in laminae I-II. On the basis of the results of the latter experiments, we chose a stimulation of 52 degrees C for 15 s to perform pharmacological investigations. The number of Fos-like immunoreactive neurons induced by the heat stimulation was significantly decreased by pretreatment with morphine (42, 64 and 75% decrease as compared to control values after 2.5, 5 and 7.5 mg/kg i.v. respectively), and these effects were blocked by naloxone. When various stimulation intensities (46-52 degrees C) were used, the effects of morphine (5 mg/kg i.v.) were most marked when the temperature was highest. In morphine-tolerant rats, morphine (5 mg/kg i.v.) was half as potent in decreasing Fos-like immunoreactivity induced by the heat stimulation than in non-tolerant rats. RB 101, a systemically active mixed inhibitor of enkephalin-metabolising enzymes, significantly decreased Fos-like immunoreactivity induced by heat stimulation (19, 29 and 48% decreases as compared to control values at 10, 20 and 40 mg/kg i.v. respectively) and these effects were blocked by naloxone. Aspirin (150 mg/kg i.v.), proacetaminophen (300 mg/kg i.v.) and tizanidine, a centrally acting myorelaxant (0.25-1 mg/kg i.v.), had no effect on the number of Fos-like immunoreactivity neurons induced by heat stimulation. The use of immunochemistry of the c-Fos protein as a pharmacological test in order to gauge antinociceptive effects at the dorsal horn level is discussed.

, **1994**
...Identifiers--ENKEPHALIN-DEGRADING ENZYMES; MIXED INHIBITOR **PRODRUG** ; **PROTEIN**-LIKE IMMUNOREACTIVITY; DORSAL HORN NEURONS; **ENDOGENOUS** ENKEPHALINS; TRANSGANGLIONIC TRANSPORT; SOMATOTOPIC ORGANIZATION; PERIPHERAL STIMULATION; TIZANIDINE DS103-282; CROSS-TOLERANCE

5/3,K,AB/5 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02813838 Genuine Article#: MF949 Number of References: 33
Title: SYNTHESIS AND ANTITUMOR-ACTIVITY OF 1-BETA-D-ARABINOFURANOSYLCYTOSINE CONJUGATES OF OPTICAL ISOMERS OF ETHER AND THIOETHER LIPIDS (Abstract Available)
Author(s): HONG CI; AN SH; NECHAEV A; KIRISITS AJ; VIG R; WEST CR
Corporate Source: ROSWELL PK CANC INST,DEPT NEUROSURG,ELM & CARLTON ST/BUFFALO//NY/14263
Journal: LIPIDS, **1993**, V28, N11 (NOV), P1021-1026
ISSN: 0024-4201
Language: ENGLISH Document Type: ARTICLE
Abstract: Four 1-beta-D-arabinofuranosylcytosine conjugates (ara-C) (1a, b and 2a, b) of sn-1 and sn-3 isomers of 1-O-octadecyl-2-O-palmitoylglycerol and its 1-S-alkyl analogue have been synthesized, and their antitumor activity against L1210 lymphoid leukemia in mice were compared with those of the previous conjugates (3a, b) of racemates in order to determine the significance of chirality of the glycerol moieties for activity. Administration (i.p.) of a single dose (300 mg/kg) of conjugates of sn-1 (1a), sn-3 (2a) and rac (3a) isomers of the ether lipid increased lifespan of i.p. implanted L1210 lymphoid leukemic DBA/2J mice by 169, 175 and 236%,

respectively. The sn-1 (1b), sn-3 (2b), and rac (3b) isomers of the thioether lipid with a single dose of 300 mg/kg produced an increase in lifespan values of 238, 263 and 250%, respectively. The results indicate that chirality of the glycerol moieties appears not to be critical for the activity, and racemates 3a and 3b are promising **prodrugs** of ara-C for further clinical investigations.

, **1993**
...Abstract: appears not to be critical for the activity, and racemates 3a and 3b are promising **prodrugs** of ara-C for further clinical investigations.
...Research Fronts: INDONESIAN MEDICINAL-PLANTS)
91-6723 001 (PHOSPHOLIPASE-A2 GENE-EXPRESSION; ARACHIDONIC-ACID RELEASE; ACTIVATION OF **PROTEIN**-KINASE-C; **ENDOGENOUS** ACTIVITY)
?

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         (c) format only 2000 Dialog Corporation
  File  55:Biosis Previews(R)  1993-2000/Jul W5
         (c) 2000 BIOSIS
  File  34:SciSearch(R) Cited Ref Sci  1990-2000/Jul W4
         (c) 2000 Inst for Sci Info
  File 434:SciSearch(R) Cited Ref Sci  1974-1989/Dec
         (c) 1998 Inst for Sci Info
  File 340:CLAIMS(R)/US Patent  1950-00/Jul 25
         (c) 2000 IFI/CLAIMS(r)

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         1449851  FACTOR
         1443908  X
      S1    7660  FACTOR(W)X
? s mutat?

      S2  517277  MUTAT?
? s s1 and s2

           7660  S1
         517277  S2
      S3     491  S1 AND S2
? s arg? or tyr?

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>>>File 34 processing for ARG? stopped at ARG4
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         283334  TYR?
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? s s3 and s4

            491  S3
         632588  S4
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Processing
Processing
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      S6     125  S5 AND PY<1998
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? s s6 and s7

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         197407  S7
      S8      54  S6 AND S7
? s enzyme

      S9  921467  ENZYME
? s s8 and s9
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        921467  S9
     S10    15  S8 AND S9
? rd

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 11/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09243282   97435318
  Interaction of factor IXa with factor VIIIa. Effects of protease domain
Ca2+ binding site, proteolysis in the autolysis loop, phospholipid, and
factor X.
  Mathur A; Zhong D; Sabharwal AK; Smith KJ; Bajaj SP
  Department of Medicine, St. Louis University School of Medicine, St.
Louis, Missouri 63104, USA.
  Journal of biological chemistry (UNITED STATES)  Sep 12 1997,  272
(37) p23418-26,  ISSN 0021-9258  Journal Code: HIV
  Contract/Grant No.: HL36365, HL, NHLBI
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  We previously identified a high affinity Ca2+ binding site in the
protease domain of factor IXa involving Glu235 (Glu70 in chymotrypsinogen
numbering; hereafter, the numbers in brackets refer to the chymotrypsin
equivalents) and Glu245[80] as putative ligands. To delineate the function
of this Ca2+ binding site, we expressed IXwild type (IXWT), IXE235K, and
IXE245V in 293 kidney cells and compared their properties with those of
factor IX isolated from normal plasma (IXNP); each protein had the same Mr
and gamma-carboxyglutamic acid content. Activation of each factor IX
protein by factor VIIa.Ca2+.tissue factor was normal as analyzed by sodium
dodecyl sulfate-gel electrophoresis. The coagulant activity of IXaWT was
approximately 93%, of IXaE235K was approximately 27%, and of IXaE245V was
approximately 4% compared with that of IXaNP. In contrast, activation by
factor XIa.Ca2+ led to proteolysis at Arg318-Ser319[150-151] in the
protease domain autolysis loop of IXaE245V with a concomitant loss of
coagulant activity; this proteolysis was moderate in IXaE235K and minimal
in IXaWT or IXaNP. Interaction of each activated mutant with an active site
probe, p-aminobenzamidine, was also examined; the Kd of interaction in the
absence and presence (in parentheses) of Ca2+ was: IXaNP or IXaWT 230
microM (78 microM), IXaE235K 150 microM (145 microM), IXaE245V 225 microM
(240 microM), and autolysis loop cleaved IXaE245V 330 microM (350
microM). Next, we evaluated the apparent Kd (Kd,app) of interaction of each
activated mutant with factor VIIIa. We first investigated the EC50 of
interaction of IXaNP as well as of IXaWT with factor VIIIa in the presence
and absence of phospholipid (PL) and varying concentrations of factor
X. At each factor X concentration and constant factor
VIIIa, EC50 was the free IXaNP or IXaWT concentration that yielded a
half-maximal rate of factor Xa generation. EC50 values for IXaNP and IXaWT
were similar and are as follows: PL-minus/X-minus (extrapolated), 2.8
microM; PL-minus/X-saturating, 0.25 &mgr;M; PLplus/X-minus, 1.6 nM; and
PL-plus/X-saturating, 0.09 nM. Further, Kd,app of binding of active
site-blocked factor IXa to factor VIIIa was calculated from its ability to
inhibit IXaWT in the Tenase assay. Kd,app values in the absence and
presence (in parentheses) of PL were: IXaNP or IXaWT, 0. 19 microM (0.07
nM); IXaE235K, 0.68 microM (0.26 nM); IXaE245V, 2.5 microM (1.35 nM); and
autolysis loop-cleaved IXaE245V, 15.6 microM (14.3 nM). We conclude
```

that (a) PL increases the apparent affinity of factor IXa for factor VIIIa approximately 2,000-fold, and the substrate, **factor X** , increases this affinity approximately 10-15-fold; (b) the protease domain Ca2+ binding site increases this affinity approximately 15-fold, and lysine at position 235 only partly substitutes for Ca2+; (c) Ca2+ binding to the protease domain increases the S1 reactivity approximately 3-fold and prevents proteolysis in the autolysis loop; and (d) proteolysis in the autolysis loop leads to a loss of catalytic efficiency with retention of S1 binding site and a further approximately 8-fold reduction in affinity of factor IXa for factor VIIIa.

... VIIIa. Effects of protease domain Ca2+ binding site, proteolysis in the autolysis loop, phospholipid, and **factor X**.
Sep 12 **1997**,
... with that of IXaNP. In contrast, activation by factor XIa.Ca2+ led to proteolysis at **Arg318** -Ser319[150-151] in the protease domain autolysis loop of IXaE245V with a concomitant loss...
... 78 microM), IXaE235K 150 microM (145 microM), IXaE245V 225 microM (240 microM), and autolysis loop **cleaved** IXaE245V 330 microM (350 microM). Next, we evaluated the apparent Kd (Kd,app) of interaction...

... with factor VIIIa in the presence and absence of phospholipid (PL) and varying concentrations of **factor X**. At each **factor X** concentration and constant factor VIIIa, EC50 was the free IXaNP or IXaWT concentration that yielded...

...68 microM (0.26 nM); IXaE245V, 2.5 microM (1.35 nM); and autolysis loop-**cleaved** IXaE245V, 15.6 microM (14.3 nM). We conclude that (a) PL increases the apparent affinity of factor IXa for factor VIIIa approximately 2,000-fold, and the substrate, **factor X** , increases this affinity approximately 10-15-fold; (b) the protease domain Ca2+ binding site increases...
; Benzamidines--Metabolism--ME; Binding Sites; Binding, Competitive; Calcium--Metabolism--ME; **Enzyme** Activation; Factor IXa--Genetics--GE; Factor VIIa--Pharmacology--PD; **Factor X**--Pharmacology--PD; Factor XIa--Pharmacology--PD; Models, Chemical; Models, Molecular; **Mutation**; Peptide Fragments--Pharmacology--PD; Phospholipids --Pharmacology--PD; Protein Binding--Drug Effects--DE; Sequence Analysis; 1 ...
...Chemical Name: IXa; (Factor XIa; (Benzamidines; (Peptide Fragments; (Phospholipids; (4-aminobenzamidine; (1-Carboxyglutamic Acid; (Factor VIIIa; (Calcium; (**Factor X**

11/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08879549 97094811
Factor VII central. A novel **mutation** in the catalytic domain that reduces tissue factor binding, impairs activation by factor Xa, and abolishes amidolytic and coagulant activity.
Bharadwaj D; Iino M; Kontoyianni M; Smith KJ; Foster DC; Kisiel W
Department of Pathology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131, USA.
Journal of biological chemistry (UNITED STATES) Nov 29 **1996**, 271 (48) p30685-91, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: HL35246, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Factor VII is a vitamin K-dependent zymogen of a serine protease that participates in the initial phase of blood coagulation. A factor VII molecular variant (factor VII Central) was identified in a 24-year-old male with severe factor VII deficiency and whose plasma factor VII antigen was 38% of normal, but expressed <1% factor VII procoagulant activity. DNA

sequence analysis of the patient's factor VII gene revealed a thymidine to cytidine transition at nucleotide 10907 in exon VIII that results in a novel amino acid substitution of Phe328 to Ser. The patient was homozygous for this **mutation** , whereas each parent of the patient was heterozygous for this **mutation** . To investigate the molecular properties of this variant, a recombinant F328S factor VII mutant was prepared and analyzed in relation to wild-type factor VII. F328S factor VII exhibited <1% factor VII procoagulant activity and a 2-fold decreased affinity for tissue factor and failed to activate **factor X** or IX in the presence of tissue factor following activation by factor Xa. In addition, F328S factor VIIa exhibited no detectable amidolytic activity in the presence of tissue factor. The rate of F328S factor VII activation by factor Xa was markedly decreased relative to the rate of wild-type factor VII activation as revealed by densitometry scanning of SDS gels. Temporal analysis of this reaction by SDS-polyacrylamide gel electrophoresis also revealed the formation of two novel F328S factor VII degradation products (40 and 9 kDa) resulting from factor Xa proteolysis of the **Arg315** -Lys316 peptide bond in intact F328S factor VII. Computer modeling and molecular dynamics simulations of the serine protease domain of factor VIIa suggested that the inability of F328S factor VIIa to **cleave** substrates may result from the apparent formation of a hydrogen bond between **Tyr377** and Asp338, a residue at the bottom of the substrate-binding pocket important for the interaction of substrate **arginine** side chains with the **enzyme**. These findings suggest that Phe328, which is conserved in prothrombin, factor IX, **factor X**, factor VII, and trypsin, is important for factor VIIa catalysis.

Factor VII central. A novel **mutation** in the catalytic domain that reduces tissue factor binding, impairs activation by factor Xa, and...

Nov 29 **1996**,

... a novel amino acid substitution of Phe328 to Ser. The patient was homozygous for this **mutation**, whereas each parent of the patient was heterozygous for this **mutation** . To investigate the molecular properties of this variant, a recombinant F328S factor VII mutant was...

... procoagulant activity and a 2-fold decreased affinity for tissue factor and failed to activate **factor X** or IX in the presence of tissue factor following activation by factor Xa. In addition...

... factor VII degradation products (40 and 9 kDa) resulting from factor Xa proteolysis of the **Arg315**-Lys316 peptide bond in intact F328S factor VII. Computer modeling and molecular dynamics simulations of...

... serine protease domain of factor VIIa suggested that the inability of F328S factor VIIa to **cleave** substrates may result from the apparent formation of a hydrogen bond between **Tyr377** and Asp338, a residue at the bottom of the substrate-binding pocket important for the interaction of substrate **arginine** side chains with the **enzyme**. These findings suggest that Phe328, which is conserved in prothrombin, factor IX, **factor X**, factor VII, and trypsin, is important for factor VIIa catalysis.

...; Chemistry--CH; Factor VII--Metabolism--ME; Factor Xa--Metabolism --ME; Hydrogen Bonding; Models, Molecular; Point **Mutation**; Recombinant Proteins; Structure-Activity Relationship; Thromboplastin --Metabolism--ME; **Tyrosine**--Chemistry--CH

Chemical Name: Factor Xa; (Recombinant Proteins; (**Tyrosine**; (Aspartic Acid; (Factor VII; (Thromboplastin

11/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08231624 95101458

Factor IX Bm Kiryu: a Val-313-to-Asp substitution in the catalytic domain results in loss of function due to a conformational change of the surface

loop: evidence obtained by chimaeric modelling.

Miyata T; Kuze K; Matsusue T; Komooka H; Kamiya K; Umeyama H; Matsui A; Kato H; Yoshioka A

Laboratory of Thrombosis Research, National Cardiovascular Centre Research Institute, Fujishirodai, Japan.

British journal of haematology (ENGLAND) Sep **1994**, 88 (1) p156-65, ISSN 0007-1048 Journal Code: AXC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Factor IX Kiryu is a naturally occurring mutant of factor IX that has 2.5% coagulant activity, even though normal plasma levels of factor IX antigen are detected. Factor IX Kiryu was purified from a patient's plasma by immunoaffinity chromatography with a calcium-dependent anti-factor IX monoclonal antibody column. It was **cleaved** normally by factor XIa in the presence of Ca2+, yielding a two-chain factor IXa. However, the resulting factor IXa showed only 1.5% of the normal factor IXa in terms of **factor X** activation in the presence of factor VIII, phospholipids, and Ca2+, and had 20% of the normal esterase activity for Z-**Arg**-p-nitrobenzyl ester. Therefore factor IXa Kiryu showed the defect of the catalytic triad or primary substrate binding site as well as defective interaction with factors VIII/X. Single-strand conformational polymorphism analysis and DNA sequencing of the amplified DNA revealed a missense point **mutation**, a T-to-A substitution at nucleotide number 31,059 of the factor IX Kiryu gene. This **mutation** resulted in the amino acid substitution of Val-313 by Asp in the catalytic domain. Restriction **enzyme** analysis of the amplified DNA showed that the **mutation** was inherited from the patient's mother. The chimaeric method was employed to construct a model of the serine protease domain of factor IXa, and the resultant model suggested that the Val-313 to Asp substitution altered the conformation of the substrate-binding site. These data combined with our previous findings on a Gly-311-to-Glu mutant of factor IX suggest that the loop conformation from Gly-311 to **ARg**-318 is important for the expression of coagulant activity.

Sep **1994**,

... by immunoaffinity chromatography with a calcium-dependent anti-factor IX monoclonal antibody column. It was **cleaved** normally by factor XIa in the presence of Ca2+, yielding a two-chain factor IXa...

... resulting factor IXa showed only 1.5% of the normal factor IXa in terms of **factor X** activation in the presence of factor VIII, phospholipids, and Ca2+, and had 20% of the normal esterase activity for Z-**Arg**-p-nitrobenzyl ester. Therefore factor IXa Kiryu showed the defect of the catalytic triad or...

... strand conformational polymorphism analysis and DNA sequencing of the amplified DNA revealed a missense point **mutation** , a T-to-A substitution at nucleotide number 31,059 of the factor IX Kiryu gene. This **mutation** resulted in the amino acid substitution of Val-313 by Asp in the catalytic domain. Restriction **enzyme** analysis of the amplified DNA showed that the **mutation** was inherited from the patient's mother. The chimaeric method was employed to construct a...

... to-Glu mutant of factor IX suggest that the loop conformation from Gly-311 to **ARg** -318 is important for the expression of coagulant activity.

Descriptors: Factor IXa--Genetics--GE; *Hemophilia B--Genetics--GE; * **Mutation**

11/3,K,AB/4 (Item 4 from file: 155)

07859981 94043230

Factor IX Fukuoka. Substitution of ASN92 by His in the second epidermal growth factor-like domain results in defective interaction with factors VIIa/X.

Nishimura H; Takeya H; Miyata T; Suehiro K; Okamura T; Niho Y; Iwanaga S
Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan.

Journal of biological chemistry (UNITED STATES) Nov 15 **1993**, 268 (32) p24041-6, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Hemophilia B Fukuoka, a moderately severe bleeding disorder, is a naturally occurring mutant of factor IX. Plasma from our patient had 3% clotting activity even though 64% of factor IX antigen was present. The purified mutant protein was **cleaved** normally by factor Xla, factor VIIa-tissue factor complex, or RVV-X (**factor X**-activating **enzyme** from Russell's viper venom), yielding a two-chain factor IXa. Amino acid composition and sequence analyses of one of the lysyl endopeptidase peptides derived from factor IX Fukuoka revealed that Asn92 in the second epidermal growth factor (EGF)-like domain had been replaced by His. The active site of the factor IXa Fukuoka was normally competent for the incorporation of p-aminobenzamidine and for the hydrolysis of a synthetic substrate, N alpha-benzyloxycarbonyl-L-**arginine** p-nitrobenzyl ester. Factor Xa formation by factor IXa Fukuoka was only 8% of the normal factor IXa, even in the presence of polylysine, and only 0.2% of the normal in the system containing phospholipids, Ca2+, and factor VIIIa, thereby indicating a functional defect in interaction of the mutant with factors VIIIa/X. Furthermore, catalytic efficiency (kcat/Km) of factor IXa Fukuoka toward **factor X** in the presence of Ca2+, phospholipids, and factor VIIIa was only 2.3% of the normal factor IXa. These results suggest that an Asn-to-His substitution at position 92 in the second EGF-like domain of factor IX Fukuoka would have an untoward effect on the specific conformational state of factor IX for binding with factors VIIIa/X.

Nov 15 **1993**,
... activity even though 64% of factor IX antigen was present. The purified mutant protein was **cleaved** normally by factor Xla, factor VIIa-tissue factor complex, or RVV-X (**factor X**-activating **enzyme** from Russell's viper venom), yielding a two-chain factor IXa. Amino acid composition and...

...of p-aminobenzamidine and for the hydrolysis of a synthetic substrate, N alpha-benzyloxycarbonyl-L-**arginine** p-nitrobenzyl ester. Factor Xa formation by factor IXa Fukuoka was only 8% of the...

...mutant with factors VIIIa/X. Furthermore, catalytic efficiency (kcat/Km) of factor IXa Fukuoka toward **factor X** in the presence of Ca2+, phospholipids, and factor VIIIa was only 2.3% of the...

Descriptors: Factor IX--Genetics--GE; *Factor VIIa--Metabolism--ME; *Factor X--Metabolism--ME; *Hemophilia B--Genetics--GE...; Metabolism--ME; Factor IX--Metabolism--ME; Hemophilia B--Metabolism--ME; Histidine--Genetics--GE; Hydrolysis; Kinetics; **Mutation**; Peptide Mapping

...Chemical Name: Factor VIIa; (Benzamidines; (Blood Coagulation Factors; (4-aminobenzamidine; (Epidermal Growth Factor; (Asparagine; (Histidine; (Factor IX; (**Factor X**

11/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06581232 90147571
Replacement of isoleucine-397 by threonine in the clotting proteinase factor IXa (Los Angeles and Long Beach variants) affects macromolecular

catalysis but not L-tosylarginine methyl ester hydrolysis. Lack of correlation between the ox brain prothrombin time and the **mutation** site in the variant proteins.

Spitzer SG; Warn-Cramer BJ; Kasper CK; Bajaj SP
Department of Medicine, St. Louis University School of Medicine, MO 63104.

Biochemical journal (ENGLAND) Jan 1 **1990**, 265 (1) p219-25,
ISSN 0264-6021 Journal Code: 9YO
Contract/Grant No.: HL36365, HL, NHLBI; HL30572, HL, NHLBI; HL07050, HL, NHLBI; +
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Previously, from the plasma of unrelated haemophilia-B patients, we isolated two non-functional Factor IX variants, namely Los Angeles (IXLA) and Long Beach (IXLB). Both variants could be **cleaved** to yield Factor IXa-like molecules, but were defective in catalysing the **cleavage** of **Factor X** (macromolecular substrate) and in binding to antithrombin III (macromolecular inhibitor). In the present study we have identified the **mutation** of IXLA by amplifying the exons (including flanking regions) as well as the 5' end of the gene by polymerase-chain-reaction (PCR) method and sequencing the amplified DNA by the dideoxy chain-termination method. Comparison of the normal IX and IXLA sequences revealed only one base substitution (T----C) in exon VIII of IXLA, with a predicted replacement of Ile-397 to Thr in the mature protein. This **mutation** is the same as found recently for IXLB. The observation that IXLB and IXLA have the same **mutation** is an unexpected finding, since, on the basis of their ox brain prothrombin time (PT, a test that measures the ability of the variant Factor IX molecules to inhibit the activation of **Factor X** by Factor VIIa-tissue factor complex), these variants have been classified into two different groups and were thought to be genetically different. Our observation thus suggests that the ox brain PT does not reflect the locus of **mutation** in the coding region of the variant molecules. However, our analysis suggests that the ox brain PT is related to Factor IX antigen concentration in the patient's plasma. Importantly, although the **mutation** in IXLA or IXLB protein is in the catalytic domain, purified IXaLA and IXaLB hydrolyse L-tosylarginine methyl ester at rates very similar to that of normal IXa. These data, in conjunction with our recent data on Factor IXBm Lake Elsinore (Ala-390----Val mutant), strengthen a conclusion that the peptide region containing residues 390-397 of normal Factor IXa plays an essential role in macromolecular substrate catalysis and inhibitor binding. However, the two **mutations** noted thus far in this region do not distort S1 binding site in the Factor IXa **enzyme**.

...tosylarginine methyl ester hydrolysis. Lack of correlation between the ox brain prothrombin time and the **mutation** site in the variant proteins.
Jan 1 **1990**,

... Factor IX variants, namely Los Angeles (IXLA) and Long Beach (IXLB). Both variants could be **cleaved** to yield Factor IXa-like molecules, but were defective in catalysing the **cleavage** of **Factor X** (macromolecular substrate) and in binding to antithrombin III (macromolecular inhibitor). In the present study we have identified the **mutation** of IXLA by amplifying the exons (including flanking regions) as well as the 5' end...

... IXLA, with a predicted replacement of Ile-397 to Thr in the mature protein. This **mutation** is the same as found recently for IXLB. The observation that IXLB and IXLA have the same **mutation** is an unexpected finding, since, on the basis of their ox brain prothrombin time (PT...
... that measures the ability of the variant Factor IX molecules to inhibit the activation of **Factor X** by Factor VIIa-tissue factor complex), these variants have been classified into two different groups...

... Our observation thus suggests that the ox brain PT does not reflect the locus of **mutation** in the coding region of the variant molecules. However, our analysis suggests that the ox...

... is related to Factor IX antigen concentration in the patient's plasma. Importantly, although the **mutation** in IXLA or IXLB protein is in the catalytic domain, purified IXaLA and IXaLB hydrolyse...

... IXa plays an essential role in macromolecular substrate catalysis and inhibitor binding. However, the two **mutations** noted thus far in this region do not distort S1 binding site in the Factor IXa **enzyme**.

Descriptors: **Arginine**--Analogs and Derivatives--AA; *Factor IXa --Genetics--GE; *Isoleucine--Genetics--GE; *Prothrombin Time; *Threonine --Genetics...
...; Esterases--Metabolism--ME; Factor IX--Immunology--IM; Factor IXa --Metabolism--ME; Hydrolysis; Molecular Sequence Data; **Mutation**; Polymerase Chain Reaction; Restriction Mapping

Chemical Name: Esterases; (Factor IXa; (Antigens; (Isoleucine; (**Arginine**; (Threonine; (Factor IX; (DNA; (Tosylarginine Methyl Ester

11/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06348992 90078229

Blood clotting factor IX BM Nagoya. Substitution of **arginine** 180 by tryptophan and its activation by alpha-chymotrypsin and rat mast cell chymase.

Suehiro K; Kawabata S; Miyata T; Takeya H; Takamatsu J; Ogata K; Kamiya T ; Saito H; Niho Y; Iwanaga S

First Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Journal of biological chemistry (UNITED STATES) Dec 15 **1989**, 264 (35) p21257-65, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Factor IX BM Nagoya (IX Nagoya) is a natural mutant of factor IX responsible for severe hemophilia B. A patient with this mutant is characterized by a markedly prolonged ox brain prothrombin time. IX Nagoya was purified from the patient's plasma by immunoaffinity chromatography with an anti-factor IX monoclonal antibody column. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that treatment of IX Nagoya with factor XIa/Ca2+ resulted in **cleavage** only at the **Arg145** -Ala146 bond. Reversed-phase high performance liquid chromatography of a trypsin digest of IX Nagoya showed an aberrant peptide, which was further digested with proteinase Asp-N. Primary structure analysis of one of the Asp-N peptides revealed that **Arg180** is replaced by Trp. An essentially complete (99%) amino acid sequence of IX Nagoya was obtained by sequencing fragments derived from a lysyl endopeptidase digest in which no other substitutions in the catalytic triad or substrate binding site were found. We also found that IX Nagoya is activated by alpha-chymotrypsin or rat mast cell chymase by monitoring the rate of **factor X** activation using a fluorogenic peptide substrate in the presence of factor VIII, phospholipids, and Ca2+. These results indicate that the substitution of **Arg180** by Trp impairs the **cleavage** by factor XIa required for activation of this zymogen and that the substitution causes hemophilia BM.

Blood clotting factor IX BM Nagoya. Substitution of **arginine** 180 by tryptophan and its activation by alpha-chymotrypsin and rat mast cell chymase.

Dec 15 **1989**,

... polyacrylamide gel electrophoresis showed that treatment of IX Nagoya with factor XIa/Ca2+ resulted in **cleavage** only at the **Arg145**

-Ala146 bond. Reversed-phase high performance liquid chromatography of a trypsin digest of IX Nagoya...
... proteinase Asp-N. Primary structure analysis of one of the Asp-N peptides revealed that **Arg180** is replaced by Trp. An essentially complete (99%) amino acid sequence of IX Nagoya was...

... is activated by alpha-chymotrypsin or rat mast cell chymase by monitoring the rate of **factor X** activation using a fluorogenic peptide substrate in the presence of factor VIII, phospholipids, and Ca2+. These results indicate that the substitution of **Arg180** by Trp impairs the **cleavage** by factor XIa required for activation of this zymogen and that the substitution causes hemophilia...
Descriptors: **Arginine**; *Chymotrypsin--Metabolism--ME; *Factor IX --Genetics--GE; ***Mutation**; *Serine Endopeptidases--Metabolism--ME; *Tryptophan; Amino Acid Sequence; **Enzyme** Activation; Factor IX --Metabolism--ME; Kinetics; Mast Cells--Enzymology--EN; Molecular Sequence Data; Peptide Mapping...
Chemical Name: Serine Endopeptidases; (Chymotrypsin; (chymase; (factor IX BM Nagoya; (**Arginine**; (Tryptophan; (Factor IX

11/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05943186 88190085
Proteolytic requirements for thrombin activation of anti-hemophilic factor (factor VIII).
Pittman DD; Kaufman RJ
Genetics Institute, Cambridge, MA 02140.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Apr **1988**, 85 (8) p2429-33, ISSN 0027-8424
Journal Code: PV3
Contract/Grant No.: 2R44HL35946-02, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Factor VIII functions in the intrinsic pathway of coagulation as the cofactor for factor IXa proteolytic activation of **factor X**. Proteolytic **cleavage** is required for activation and may be responsible for inactivation of cofactor activity. To identify which of the multiple **cleavages** are required for activation and inactivation of factor VIII, site-directed DNA-mediated mutagenesis of the factor VIII cDNA was performed and the altered forms of factor VIII were expressed in COS-1 monkey cells and characterized. Conversion of **arginine** residues to isoleucine residues at the aminoterminal side of the **cleavage** sites at positions 740, 1648, and 1721 resulted in **cleavage** resistance at the modified site with no alteration in the in vitro procoagulant activity and the susceptibility to thrombin activation. Similar modification of the thrombin **cleavage** sites at either position 372 or position 1689 resulted in molecules with residual factor VIII activity but resistant to thrombin **cleavage** at the modified site and not susceptible to thrombin activation. Modification of the **arginine** to either an isoleucine or a lysine at residue 336, the site postulated for proteolytic inactivation by activated protein C, resulted in a factor VIII molecule with increased procoagulant activity. This increased activity may result from greater resistance to proteolytic inactivation. A model for the activation and inactivation of factor VIII is proposed.

Apr **1988**,
...in the intrinsic pathway of coagulation as the cofactor for factor IXa proteolytic activation of **factor X**. Proteolytic **cleavage** is required for activation and may be responsible for inactivation of cofactor activity. To identify which of the multiple **cleavages** are required for activation and inactivation of factor VIII, site-directed DNA-mediated mutagenesis of...

... forms of factor VIII were expressed in COS-1 monkey cells and characterized. Conversion of **arginine** residues to isoleucine residues at the aminoterminal side of the **cleavage** sites at positions 740, 1648, and 1721 resulted in **cleavage** resistance at the modified site with no alteration in the in vitro procoagulant activity and the susceptibility to thrombin activation. Similar modification of the thrombin **cleavage** sites at either position 372 or position 1689 resulted in molecules with residual factor VIII activity but resistant to thrombin **cleavage** at the modified site and not susceptible to thrombin activation. Modification of the **arginine** to either an isoleucine or a lysine at residue 336, the site postulated for proteolytic...
; Cells, Cultured; DNA **Mutational** Analysis; **Enzyme** Activation
; Molecular Weight; Structure-Activity Relationship

11/3,K,AB/8 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

8/18

06023908 Genuine Article#: XQ127 Number of References: 28
Title: Expression, purification, and characterization of recombinant human **factor X**
Author(s): Rudolph AE (REPRINT) ; Mullane MP; PorcheSorbet R; Miletich JP
Corporate Source: WASHINGTON UNIV,SCH MED, DEPT PATHOL/ST LOUIS//MO/63110 (REPRINT); WASHINGTON UNIV,SCH MED, DEPT MED, DIV LAB MED/ST LOUIS//MO/63110
Journal: PROTEIN EXPRESSION AND PURIFICATION, **1997**, V10, N3 (AUG), P 373-378
ISSN: 1046-5928 Publication date: 19970800
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495
Language: English Document Type: ARTICLE
Abstract: A system is described for producing recombinant **factor X** with properties very similar to human plasma **factor X**, Optimization of the expression system for **factor X** resulted in the finding that human kidney cells (293 cells) are superior to the widely utilized baby hamster kidney cells (BHK cells) for the expression of functional **factor X**, It was also determined that production of **factor X** by 293 cells requires the substitution of the -2 residue (Thr --> **Arg**) which affords the removal of the **factor X** propeptide, Purification of recombinant and plasma **factor X** is accomplished using a calcium-dependent monoclonal antibody directed against the gla domain, The proteins are comparable by sodium dodecyl sulfate polyacrylamide gel electrophoresis, The rate and extent of activation by the **factor X** coagulant protein from Russell's viper venom and by factors Ma and VIIIa are similar; activation of the recombinant protein by VIIa and tissue factor is mildly faster, The activated enzymes have the same activity toward a chromogenic substrate and the biologic substrate, prothrombin, Both enzymes leave the same apparent affinity for the activated platelet surface as judged by their ability to activate prothrombin, Finally, inhibition by antithrombin, with or without heparin, and inhibition by the tissue factor pathway inhibitor are equivalent, Recombinant **factor X** produced by this method is therefore well suited for probing structure-function relationships by **mutational** analysis, (C) 1997 Academic Press.

Title: Expression, purification, and characterization of recombinant human **factor X**
, **1997**
Abstract: A system is described for producing recombinant **factor X** with properties very similar to human plasma **factor X**, Optimization of the expression system for **factor X** resulted in the finding that human kidney cells (293 cells) are

superior to the widely utilized baby hamster kidney cells (BHK cells) for the expression of functional **factor X**, It was also determined that production of **factor X** by 293 cells requires the substitution of the -2 residue (Thr --> **Arg**) which affords the removal of the **factor X** propeptide, Purification of recombinant and plasma **factor X** is accomplished using a calcium-dependent monoclonal antibody directed against the gla domain, The proteins...

...by sodium dodecyl sulfate polyacrylamide gel electrophoresis, The rate and extent of activation by the **factor X** coagulant protein from Russell's viper venom and by factors Ma and VIIIa are similar...

...with or without heparin, and inhibition by the tissue factor pathway inhibitor are equivalent, Recombinant **factor X** produced by this method is therefore well suited for probing structure-function relationships by **mutational** analysis, (C) 1997 Academic Press.
...Identifiers--COAGULATION FACTOR-VII; RUSSELLS VIPER VENOM; ENDOPLASMIC-RETICULUM; ANTITHROMBIN-III; HUMAN PLATELETS; ACTIVATION; MECHANISM; **CLEAVAGE**; HEPARIN; **ENZYME**
...Research Fronts: VENOUS THROMBOSIS; FACTOR-V LEIDEN)
95-3190 001 (INCREASED ABUNDANCE OF SPECIFIC SKELETAL-MUSCLE PROTEIN-**TYROSINE** PHOSPHATASES; ALPHA-B-CRYSTALLIN EXPRESSION)
95-3491 001 (TISSUE FACTOR PATHWAY INHIBITOR; INTEGRIN REGULATION OF...

11/3,K,AB/9 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05213000 Genuine Article#: VJ442 Number of References: 41
Title: ROLE OF RESIDUE-99 AT THE S2-SUBSITE OF FACTOR-XA AND ACTIVATED PROTEIN-C IN **ENZYME** SPECIFICITY
Author(s): REZAIE AR
Corporate Source: OKLAHOMA MED RES FDN,CARDIOVASC BIOL RES PROGRAM,825 NE 13TH ST/OKLAHOMA CITY//OK/73104
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, **1996**, V271, N39 (SEP 27), P 23807-23814
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Abstract: It is thought that only a limited number of residues in the extended binding pocket of coagulation proteases are critical for substrate and inhibitor specificity, A candidate residue from the crystal structures of thrombin and factor Xa (FXa) that may be critical for specificity at the S2 subsite is residue 99, Residue 99 is **Tyr** in FXa and Thr in activated protein C (APC), To determine the role of residue 99 in S2 specificity, a Gla-domainless mutant of protein C (GDPC) was prepared in which Thr(99) was replaced with **Tyr** of FXa, GDPC T99Y bound Ca2+ and was activated by the thrombin-thrombomodulin complex normally, The T99Y mutant, similar to FXa, hydrolyzed the chromogenic substrates with a Gly at the P2 positions, This mutant was also inhibited by antithrombin (AT) (k(2) = 4.2 +/- 0.2 x 10(1) M(-1) s(-1)), and heparin accelerated the reaction >350-fold (k(2) = 1.5 +/- 0.1 x 10(4) M(-1) s(-1)). The T99Y mutant, however, did not activate prothrombin but inactivated factor Va approximate to 2-fold better than wild type, To try to switch the specificity of FXa, both **Tyr**(99) and Gln(192) of FXa were replaced with those of APC in the Gla-domainless **factor X** (GDFX Y99T/Q192E), This mutant was folded correctly as it bound Ca2+ with a similar affinity as GDFX and was also activated by the Russell's viper venom at similar rate, but it **cleaved** the chromogenic substrates with a Gly at the P2 positions poorly, The mutant, instead, **cleaved** the APC-specific chromogenic substrates efficiently, The Y99T/Q192E mutant became resistant to inhibition by AT in the absence of heparin but was inhibited by AT almost normally in the presence of

heparin (k(2) = 3.4 +/- 0.5 x 10(5) M(-1) s(-1)). The Y99T/Q192E mutant did not inactivate factor Va, and prothrombin activation by this mutant was impaired, These results indicate that 1) residue 99 is critical for **enzyme** specificity at the S2 subsite, 2) a role for heparin in acceleration of FXa inhibition by AT may involve the S2-P2 modulation, and 3) the exchange of residues 99 and 192 in FXa and APC may switch the **enzyme** specificity with the chromogenic substrates and inhibitors but not with the natural substrates.

...Title: OF RESIDUE-99 AT THE S2-SUBSITE OF FACTOR-XA AND ACTIVATED PROTEIN-C IN **ENZYME** SPECIFICITY

, **1996**

...Abstract: may be critical for specificity at the S2 subsite is residue 99, Residue 99 is **Tyr** in FXa and Thr in activated protein C (APC), To determine the role of residue...

...domainless mutant of protein C (GDPC) was prepared in which Thr(99) was replaced with **Tyr** of FXa, GDPC T99Y bound Ca2+ and was activated by the thrombin-thrombomodulin complex normally...

...2-fold better than wild type, To try to switch the specificity of FXa, both **Tyr**(99) and Gln(192) of FXa were replaced with those of APC in the Gla-domainless **factor X** (GDFX Y99T/Q192E), This mutant was folded correctly as it bound Ca2+ with a similar...

...and was also activated by the Russell's viper venom at similar rate, but it **cleaved** the chromogenic substrates with a Gly at the P2 positions poorly, The mutant, instead, **cleaved** the APC-specific chromogenic substrates efficiently, The Y99T/Q192E mutant became resistant to inhibition by...

...by this mutant was impaired, These results indicate that 1) residue 99 is critical for **enzyme** specificity at the S2 subsite, 2) a role for heparin in acceleration of FXa inhibition...

...3) the exchange of residues 99 and 192 in FXa and APC may switch the **enzyme** specificity with the chromogenic substrates and inhibitors but not with the natural substrates.

Research Fronts: 94-2470 002 (ACTIVATED PROTEIN-C RESISTANCE; FACTOR-V GENE **MUTATION** IN VENOUS THROMBOSIS; CAUSE OF FAMILIAL HYPERCOAGULABLE STATE)
94-2803 002 (SELECTIVE THROMBIN INHIBITORS; RECOMBINANT...

11/3,K,AB/10 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01314747 Genuine Article#: GN727 Number of References: 32
Title: FACTOR-XSANTO-DOMINGO - EVIDENCE THAT THE SEVERE CLINICAL PHENOTYPE ARISES FROM A **MUTATION** BLOCKING SECRETION
Author(s): WATZKE HH; WALLMARK A; HAMAGUCHI N; GIARDINA P; STAFFORD DW; HIGH KA
Corporate Source: UNIV N CAROLINA,DEPT MED,DIV HEMATOL,CB 7035,BURNETT WOMACK BLDG/CHAPEL HILL//NC/27599; UNIV N CAROLINA,DEPT MED,DIV HEMATOL,CB 7035,BURNETT WOMACK BLDG/CHAPEL HILL//NC/27599; UNIV N CAROLINA,DEPT PATHOL/CHAPEL HILL//NC/27599; UNIV N CAROLINA,CTR THROMBOSIS & HEMOSTASIS/CHAPEL HILL//NC/27599; CORNELL UNIV,MED CTR,COLL MED/NEW YORK//NY/10021
Journal: JOURNAL OF CLINICAL INVESTIGATION, **1991**, V88, N5, P1685-1689
Language: ENGLISH Document Type: ARTICLE
Abstract: **Factor X** (FX) is a vitamin K-dependent plasma protein required for the intrinsic and extrinsic pathways of blood coagulation. FX(Santo Domingo) is a hereditary FX deficiency which is characterized clinically by a severe bleeding diathesis. The proposita has a FX activity of < 1% and a FX antigen of < 5%. We have determined the molecular basis of the defect in the FX(Santo Domingo) gene by

amplification of all eight exons with polymerase chain reaction and subsequent sequence analysis. The patient is homozygous for a G --> A transition in exon I at codon -20 (numbering the alanine at the NH, terminus of the mature protein as +1), resulting in the substitution of **arginine** for glycine in the carboxy-terminal part of the signal peptide. This amino acid change occurs near the presumed **cleavage** site of the signal peptidase. We hypothesized that the **mutation** might prevent **cleavage** by the signal peptidase which in turn would impair proper secretion of the FX protein. To test this hypothesis, we compared the expression of wild type and mutant FX cDNA in a human kidney cell line. Wild type and mutant constructs in the expression vector pCMV4 were introduced into the human embryonic kidney cell line 293 by calcium phosphate transfection. FX antigen levels in the supernatant of the cells harboring the wild type construct were 2.4-mu-g/10(7) cells per 24 h, whereas antigen levels in media from cells containing the FX(Santo Domingo) construct were undetectable. No FX antigen was detected in the cell lysates of cells transfected with the mutant construct. To insure that the difference in protein levels was not due to a difference in steady state levels of mRNA, Northern analysis was performed on RNA from the cell lysates of both constructs. The results showed a transcript of the same size, present in roughly equal amounts, in both cases. Thus, the defect in the signal sequence of FX(Santo Domingo) exerts its effect posttranscriptionally. FX(Santo Domingo) is the first described example of a bleeding diathesis due to a **mutation** in the signal sequence.

Title: FACTOR-XSANTO-DOMINGO - EVIDENCE THAT THE SEVERE CLINICAL PHENOTYPE ARISES FROM A **MUTATION** BLOCKING SECRETION
, **1991**
Abstract: **Factor X** (FX) is a vitamin K-dependent plasma protein required for the intrinsic and extrinsic pathways...

...at the NH, terminus of the mature protein as +1), resulting in the substitution of **arginine** for glycine in the carboxy-terminal part of the signal peptide. This amino acid change occurs near the presumed **cleavage** site of the signal peptidase. We hypothesized that the **mutation** might prevent **cleavage** by the signal peptidase which in turn would impair proper secretion of the FX protein...

...FX(Santo Domingo) is the first described example of a bleeding diathesis due to a **mutation** in the signal sequence.
...Identifiers--BLOOD-COAGULATION FACTOR; HUMAN **FACTOR-X**; **CLEAVAGE** SITE; MOLECULAR DEFECT; SIGNAL PEPTIDE; GENE; DNA; SEQUENCE; PROTEIN; **ENZYME**
...Research Fronts: AMPLIFIED GENOMIC DNA; DIRECT SEQUENCING)
89-5619 001 (FACTOR-IX GENE; DIRECT DETECTION OF POINT **MUTATIONS**; VITAMIN-K-DEPENDENT PROTEINS; PROPEPTIDE **CLEAVAGE**; AMINO-ACID SUBSTITUTION)
89-5918 001 (PROTEIN EXPRESSION VIA A CIS-ACTING SEQUENCE; VIRAL PROMOTER...

...7767 001 (SIGNAL SEQUENCE; ESCHERICHIA-COLI LEADER PEPTIDASE; MOLECULAR-CLONING OF HUMAN TESTICULAR ANGIOTENSIN-CONVERTING **ENZYME**)